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University of Helsinki and Helsinki University Hospital

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DEVELOPMENT OF PAIRED ASSOCIATIVE STIMULATION FOR MOTOR REHABILITATION IN SPINAL CORD INJURY PATIENTS

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DOCTORAL DISSERTATION

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Helsinki 2021

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PREFACE

I remember very well how I started my life in Finland. 18 August 2014, on the very first working day in the Biomag laboratory, I was shown my room where another young woman was already sitting. It was Anastasia Shulga. As chance would have it, this was also her very first day as a postdoctoral researcher in Biomag. The first 1.5 years I had been working for another project and I was going to continue so, if…if MEG would not go for many-month upgrading. Naturally, Anastasia, as the person sitting next to me at work, witnessed all my indignation at this MEG-off situation. Ironically, at that time she was leaving Biomag for her medical practice for half a year and she was thinking about who might replace her in the ongoing patient stimulations. One could easily guess who it was! She proposed interesting work to me and I gladly accepted it. And so, a chain of events brought me to the spinal cord injury project and where I was Anastasia's first PhD student.

I have to say that I opened the world of research just when I started to work at Biomag; before I was only doing medical practice. As any beginner, I was struggling a lot. I was lucky to have such a supervisor and a fellow as Anastasia. In fact, my confidence as a scientist grew mostly due to Anastasia's assistance. She was always kind and supportive, and, importantly, fair minded. I send a separate thanks for her patience. Overall, she became for me an example of how to manage a team and this is what I will aim for in my career. I also thank her for being my friend outside of work.

I was also lucky to have Jyrki Mäkelä as my other supervisor. He was also very kind and not only gave me wise advice but at the same time also gave me freedom to make my own decisions. I learned from him how to be diplomatic and stay calm in complicated situations.

There are a lot of people I wish to thank. I am thankful to Pantelis Lioumis for creating a good mood and being positive, for our interesting conversations, and for being always ready to help me. I am thankful to Andrei Zhdanov for our fascinating conversations in the Biomag kitchen. If I needed help in resolving a complicated matter, Andrei's critical approach to everything just made an excellent work! I thank Juha Montonen for always being responsive to any issue. He introduced a good tradition of bringing fresh ground fancy coffee to Biomag, so no one wants come back to Juhla Mokka anymore! I thank Hanna Renvall, the current head of Biomag laboratory, for creating an atmosphere of friendship. I believe that it is hard to imagine a better workplace than the Biomag laboratory.

I thank all people with whom we performed research. Erika Haaksiluoto assisted in all patient studies. Her solid experience helped in managing the difficult clinical cases. From her I learned the basics of neurophysiological measurements. I thank Sarianna Savolainen for staying with us at all patient studies and for performing patient evaluations.

I am deeply thankful to my patients for their multi-week participation. Seeing how they improved during treatment, not the publications, made me truly happy and motivated!

I am thankful to my friends Ilida Suleymanova and Alexey Pospelov who were always next to me sharing the good and bad moments.

It was a coincidence that the hard time of the coronavirus pandemic dropped upon us at the moment when I had already finished all experiments and just had to write my dissertation. That was a year when I could stay at work in silence being wrapped up in writing and reading. Hopefully, the work is done and the world is coming back to normal life!

I want to say thank to Eini-Marie Majavirta. She held up my chin at a critical period in my life and ultimately became my friend.

Thanks to my family. For performing self-organization at work, thanks to my beloved son Pietro since I could not stay late at Biomag and had to run home to see him. A big thanks to my husband Flavio. He always supported me and believed in me. Without him I could not complete doctoral studies this fast. Thanks to my parents for their unconditional love.

Thanks to the Finnish Cultural Foundation, Doctoral Program in Health Science of University of Helsinki, and Academy of Finland, which provided full financial support for my doctoral studies.

CONTENTS

TIIVISTELMÄ

Selkäydinvamma (SYV) on toimintakykyä heikentävä tila, jolla on suuri sosioekonominen vaikutus sekä vammautuneeseen henkilöön että terveydenhuoltojärjestelmään. Koska SYVin akuuttihoito on parantunut huomattavasti, myös kuntoutukselle on suuri tarve potilaiden elämänlaadun parantamiseksi. SYVissa hermoimpulssien kulku aivoista kohde-elimiin ja takaisin on estynyt, mikä voi johtaa mm. raajojen paralyysiin. Tällä hetkellä SYViin ei ole parantavaa hoitoa. Laajassa käytössä olevat tavanomaisen kuntoutuksen menetelmät eivät palauta riittävästi liikuntakykyä erityisesti vaikean SYVin jälkeen. Synkronoitu sähkö- ja magneettistimulaatio (PAS, paired associative stimulation) on suhteellisen uusi kajoamaton menetelmä, jossa kahta kohdetta motorisessa järjestelmässä aktivoidaan samanaikaisesti. Yksittäinen PAS-kerta tehostaa ohimenevästi motoristen ratojen yhteyksiä. Useiden viikkojen aikana annettavan PASin terapeuttista potentiaalia neurologisten potilaiden merkityksellisen toimintakyvyn kohenemisessa ei tunneta.

Tämän väitöskirjan tarkoituksena on tutkia pitkäaikaisen PASin vaikutuksia käden motoriikan paranemiseen kroonisilla SYV potilailla (osatyöt IV, V, VI). PAS stimulaatioprotokollaa on myös parannettu SYVin jälkeisten keskushermoston toiminnallisia muutoksia huomioiden (osatyöt I, II, III).

PAS koostuu transkraniaalisesta magneettistimulaatiosta (TMS) ja perifeerisesta sähköstimulaatiosta (PNS, peripheral nerve stimulation). TMS:n ja PNS:n synnyttämät neuroniaktivaatiot on ajoitettu kohtaamaan selkäydintasolla, mikä johtaa liikeratojen toimintaa parantavaan LTP (long term potentiation) -ilmiöön ylempien ja alempien motoneuronien välisissä synapseissa. Optimaalinen ajoitus määritellään laskemalla TMS:n ja PNS:n välinen aika eli ISI (interstimulus interval). Ns ”klassinen” PAS-protokolla vaatii tarkan ajoituksen jotta LTP ilmiö syntyisi. Olemme muokanneet klassista PAS protokollaa (käyttäen TMSaa korkealla intensiteetillä ja PNSaa korkealla taajuudella) jotta PAS olisi tehokkaampi SYV potilailla, joilla on vammasta johtuvia muutoksia hermoradoissa.

ISIn tarkka määritys ei ole aina mahdollista SYV potilailla. Osatyössä 1 pureuduttiin tähän ongelmaan. Käytimme PAS-protokollan muunnoksia erilaisilla ISI –arvoilla. Kaikki testatut muunnokset olivat tehokkaita, mikä viittaa siihen että kehittämämme PAS protokolla on sovellettavissa neurologisille potilaille.

Osatyössä II oli kaksi koeasetelmaa. Kokeessa 2.1 haettiin optimaalisia PAS-parametreja. Testasimme PAS-muunnoksia, joissa oli käytetty erilaisia

PNS taajuuksia (25 Hz, 50 Hz, 100 Hz). Kaikki muunnokset voimistivat motorisia herätepotentiaaleja (motor-evoked potential, MEP). PAS jossa oli käytetty 100 Hz PNS asetuksia johti voimakkaimpaan ja pitkäkestoisimpaan MEP-amplitudien kasvuun. Koe 2.2 tehtiin koska liikeaivokuoren kartoitus neurologisilla potilailla on haasteellista. Osoittautui, että PAS toimii silloinkin, kun aivokuorella valittu TMS-stimulaatiopiste ei ole paras mahdollinen.

Osatyössä III oli kolme koetta. Kokeessa 3.1 haettiin protokollaa, joka olisi mahdollisimman miellyttävä potilaille. Testasimme PAS 0.4 Hz taajuutta, jonka avulla PASin kesto pystyttiin puolittamaan. Tämä lyhennetty protokolla oli tehottomampi alkuperäiseen PAS-protokollaamme (0.2 Hz) verrattuna. Kokeessa 3.2 tutkittiin korkeampien PNS taajuuksien vaikutusta PASin tehoon. PAS, jossa käytettiin 100 Hz PNS:aa oli edelleen luotettavin. Kokeessa 3.3. pyrittiin tehostamaan PAS-vaikutusta lisäämällä pulssien interaktioiden määrää selkäydintasolla käyttämällä TMS:aa 20Hz:n taajuudella. Tämä protokolla pienensi MEP-amplitudeja.

Osatyössä IV tutkittiin uuden PAS protokollan terapeuttista vaikutusta kahdella SYV-potilaalla. Osatyössä V tutkittiin PASin tehokkuutta ryhmässä potilaita, joilla on traumaattinen SYV. Neljän viikon PAS-hoidon jälkeen lihasvoima lisääntyi PAS-hoidetussa kädessä manual muscle test (MMT) -luokituksella mitattuna. Lumestimulaatio vastakkaisessa kädessä (lume-TMS ja aktiivinen PNS) lisäsi MMT-arvoja merkitsevästi vähemmän kuin oikea PAS.

Osatyössä VI käytimme pitkäaikaista PAS-hoitoa, jonka protokolla oli optimoitu terveiden koehenkilöiden mittauksissa, potilaille joilla on sairausperäinen SYV. Potilaiden MMT-arvot ja päivittäinen toimintakyky kohentuivat. Parantunut toimintakyky säilyi ainakin 6 kk hoidon lopettamisesta.

PAS-protokollamme oli suunniteltu tehostamaan säilyneitä yhteyksiä SYV-potilaiden selkäytimessä. Terveillä koehenkilöillä tehdyissä kokeissa testattiin erilaisia parametreja PAS-hoidon tehostamiseksi. Tehokkaimmat protokollat otettiin kliiniseen käyttöön. Pitkäaikaisella PASilla on terapeuttinen vaikutus, johon liittyy myös toimintakyvyn paraneminen. Pitkäaikainen PAS oli myös tehokkaampi kuin pitkäaikainen periferinen sähköstimulaatio, jota käytetään tavallisessa kuntoutuksessa. Menetelmän sopivuus, tehokkuus ja turvallisuus tekevät PASista sopivan ehdokkaan SYV-potilaiden motoriikan kuntoutukseen.

ABSTRACT

Spinal cord injury (SCI) is a debilitating condition with a considerable socioeconomic impact on healthcare resources and on the injured individuals. Since acute management of SCI has considerably improved, rehabilitation of SCI is in high demand to improve patient quality-of-life. SCI is characterized with an interruption of neuronal relay from the brain to the efferent organs and back to the brain, resulting in paralysis. Currently, there is no cure for SCI. Widely used conventional rehabilitation programs do not enable restoration of motor function in a severe SCI. Paired associative stimulation (PAS) is a relatively new non-invasive method that applies two-site stimulation within the motor system. A single PAS session results in a transient increase of motor output in neurological patients. However, the potential of long-term PAS on functionally meaningful recovery in SCI patients has not been explored.

The goal of this dissertation was to investigate the efficacy of long-term PAS on hand motor recovery in chronic SCI patients (studies IV, V, VI). The altered physiology of the motor system in SCI individuals had to be considered regarding the feasibility of the PAS protocol (studies I, II, III).

PAS was implemented with transcranial magnetic stimulation (TMS) and peripheral nerve stimulation (PNS). TMS- and PNS-induced pulses were timed to coincide in the spinal cord as determined by the value of an interstimulus interval (ISI). This neuronal interaction supposedly resulted in long-term potentiation (LTP)-like plasticity in the corticomotoneuronal synapses. A classical PAS protocol requires accurate determination of an ISI to induce LTP-like plasticity in the targeted synapses. In our laboratory, the PAS protocol was modified (a single-pulse high-intensity TMS and a high-frequency PNS train) to increase the feasibility of PAS in SCI individuals.

The exact determination of ISI is not always possible in SCI patients. Study I mimicked this clinically possible scenario. PAS protocols with different ISIs that provide non-synchronized arrival of TMS- and PNS-induced pulses were examined. All tested PAS protocols were effective, suggesting that this PAS protocol is feasible for neurological patients.

Study II consisted of two experiments. Experiment 2.1 sought to determine more effective PAS settings. PAS protocols with different frequencies of PNS train (25 Hz, 50 Hz, 100 Hz) were tested. Although all protocols increased motor-evoked potential (MEP) amplitudes, PAS with 100-Hz PNS exhibited the strongest and most sustainable MEP potentiation. Experiment 2.2 addressed the challenge of accurate motor cortex mapping in neurological

patients. We observed demonstrated efficacy of our PAS protocol even when TMS was administered to a suboptimal spot in the primary motor cortex (M1).

Study III consisted of three experiments. Experiment 3.1 sought to determine a more convenient PAS protocol for SCI patients. PAS with increased frequency of PNS-TMS pairings (0.4 Hz PAS) that allowed a half-duration PAS session was tested. The shortened protocol was less effective compared to our original PAS protocol (0.2 Hz PAS). Experiment 3.2 continued exploring the impact of PNS frequency (100 Hz, 200 Hz, 400 Hz) on the effectiveness of the PAS protocol. PAS with a 100-Hz PNS train remained the most reliable protocol. Experiment 3.3 sought to enhance PAS efficacy by increasing collision of neuronal events in the corticomotoneuronal synapses by employing 20-Hz paired-pulse TMS in PAS. This PAS protocol induced a significant MEP suppression.

Study IV assessed the efficacy of the novel PAS protocol in two subjects with SCI. Study V explored the efficacy of PAS in a group of traumatic SCI patients. After a 4-week PAS, the manual muscle testing (MMT) score improved in the PAS-treated hand. Sham PAS stimulation of the contralateral hand (sham TMS and actual PNS) induced a significantly smaller MMT score increase compared with the hand activated by PAS.

Study VI applied long-term PAS with the most effective settings (100-Hz PNS) in a group of SCI patients with different neurological origins. In addition to considerable improvement in MMT scores, daily functioning of the patients improved. The observed improvement persisted at least 6 months after the PAS treatment.

Our PAS protocol was designed to potentiate spared connections in the spinal cord in SCI patients. Modification of our PAS protocol demonstrated feasibility of long-term PAS in SCI individuals. In a series of experiments on healthy subjects, different parameters of the PAS protocol were tested with the objective of increasing PAS effectiveness. The most effective PAS protocols were translated to clinical research. Long-term PAS demonstrated a therapeutic effect that was accompanied with functional improvement. Long-term PAS outperformed long-term PNS, which is widely used in conventional rehabilitation in SCI. The feasibility, effectiveness, and safety of this method favours long-term PAS as a promising motor rehabilitation in SCI patients.

LIST OF ORIGINAL PUBLICATIONS

This doctoral dissertation consists of an overview and of the following original publications which are referred to in the text by their Roman numerals.

I. Shulga A, **Zubareva A**, Lioumis P, Makela JP. (2016). Paired associative stimulation with high-frequency peripheral component leads to enhancement of corticospinal transmission at wide range of interstimulus intervals. *Frontiers in Human Neuroscience*, 10,470. doi:10.3389/fnhum.2016.00470[doi]

II. **Tolmacheva A**, Makela JP, Shulga A. (2019). Increasing the frequency of peripheral component in paired associative stimulation strengthens its efficacy. *Scientific Reports*, 9(1), 3849-019. doi:10.1038/s41598-019-40474-0 [doi]

III. Mezes M, Havu R, **Tolmacheva A**, Lioumis P, Makela JP, Shulga A. (2020). The impact of TMS and PNS frequencies on MEP potentiation in PAS with high-frequency peripheral component. *PLoS One*, 15(5), e0233999. doi:10.1371/journal.pone.0233999 [doi].

IV. Shulga A, Lioumis P, **Zubareva A**, Brandstack N, Kuusela L, Kirveskari E, Savolainen S, Ylinen A, Makela JP. (2016). Long-term paired associative stimulation can restore voluntary control over paralyzed muscles in incomplete chronic spinal cord injury patients. *Spinal Cord Series and Cases*, 2, 16016. doi:10.1038/scsandc.2016.16 [doi]

V. **Tolmacheva A**, Savolainen S, Kirveskari E, Lioumis P, Kuusela L, Brandstack N, Ylinen A, Makela JP, Shulga A. (2017). Long-term paired associative stimulation enhances motor output of the tetraplegic hand. *Journal of Neurotrauma*, doi:10.1089/neu.2017.4996 [doi]

VI. **Tolmacheva A**, Savolainen S, Kirveskari E, Brandstack N, Makela JP, Shulga A. (2019). Paired associative stimulation improves hand function after non-traumatic spinal cord injury: A case series. *Clinical Neurophysiology Practice*, 4, 178-183. doi:10.1016/j.cnp.2019.07.002 [doi]

AUTHOR'S CONTRIBUTION

I. Paired associative stimulation with high-frequency peripheral component leads to enhancement of corticospinal transmission at wide range of interstimulus intervals.

The author conducted a part of the experiments, assisted in analysing the data, and contributed to editing of the manuscript and preparation of the final version of the manuscript.

II. Increasing the frequency of peripheral component in paired associative stimulation strengthens its efficacy.

The author participated in the study design, performed most stimulations, collected and analysed the data, wrote the first draft of the manuscript, and participated in preparation of the final version of the manuscript.

III. The impact of TMS and PNS frequencies on MEP potentiation in PAS with high-frequency peripheral component.

The author participated in designing experiments 1 and 2, supervised conduct of experiment 2, and participated in the preparation of the final version of the manuscript.

IV. Long-term paired associative stimulation can restore voluntary control over paralyzed muscles in incomplete chronic spinal cord injury patients.

The author performed half of the long-term PAS sessions and sensory score assessments. The author collected and analysed the data and participated in the preparation of the final version of the manuscript.

V. Long-term paired associative stimulation enhances motor output of the tetraplegic hand.

The author participated in designing the study, managed the logistics, and performed stimulations of all patients. The author assessed sensory scores and collected and analysed data. The author wrote the first draft of the manuscript and participated in the preparation of the final version of the manuscript.

VI. Paired associative stimulation improves hand function after non-traumatic spinal cord injury: A case series.

The author participated in designing the study. The author managed the logistics and performed stimulations of all patients. The author assessed sensory scores, mechanical hand dynamometry, and hand dexterity tests at all evaluations. The author collected and analysed the data. The author wrote the first draft of the manuscript and participated in preparation of the final version of the manuscript.

ABBREVIATIONS

ADM	abductor digiti minimi
AH	abductor hallucis brevis
APB	abductor pollicis brevis
AIS	American Spinal Injury Association impairment scale
ASIA	American Spinal Injury Association
BBT	box and block test
BDNF	brain-derived neurotrophic factor
BR	brachioradialis
CPG	central pattern generator
CSPG	chondroitin sulfate proteoglycan
CST	corticospinal tract
CT	computer tomography
E-field	electric field
EMG	electromyography/electromyogram
EPSP	excitatory postsynaptic potential
ES	electrical stimulation
FES	functional electrical stimulation
GC	gastrocnemius muscle
ICF	intracortical facilitation

ISI	interstimulus interval
ISNCSCI	International Standards for Neurological Classification of spinal cord injury
LICI	long-interval intracortical inhibition
LTD	long-term depression
LTP	long-term potentiation
M1	primary motor cortex
MEP	motor-evoked potential
MMT	manual muscle testing
MN	median nerve
MRI	magnetic resonance imaging
MSCs	mesenchymal stem cells
MSO	maximal stimulator output
PAS	paired associative stimulation
PAS/25, 50, 100, 200, 400	PAS with a PNS train of 25 Hz, 50 Hz, 100 Hz, 200 Hz, 400 Hz
PN	peroneal nerve
PNS	peripheral nerve stimulation
TMS	transcranial magnetic stimulation
nTMS	navigated transcranial magnetic stimulation
rTMS	repetitive transcranial magnetic stimulation

RMT	resting motor threshold
RN	radial nerve
ROM	range of motion
TA	tibialis anterior muscle
TN	tibial nerve
tsDCS	trans-spinal direct current stimulation
tDCS	transcranial direct current stimulation
SCI	spinal cord injury
STDP	spike-timing-dependent plasticity
UN	ulnar nerve
VAS	Visual Analog Scale
WHO	World Health Organization
ZPP	Zone of partial preservation

1. INTRODUCTION

1.1 SCI IN NUMBERS

Spinal cord injury (SCI) is a debilitating condition with considerable socioeconomic impact on affected individuals and the healthcare system. The World Health Organization (WHO) estimates that the annual global incidence of SCI is 40-80 cases per million population; 250 000 - 500 000 people suffer SCI every year with a male-to-female ratio of approximately 2:1. Age distribution peaks are in young adulthood and in those > 60 years, reflecting the leading causes of SCI. Vehicular accidents are responsible for approximately 40% of SCI and are characteristic for young adults, whereas falls (approximately 32%) are the main cause of SCI in the elderly population. Most studies on SCI have focused on traumatic patients; the incidence of non-traumatic SCI is highly variable as existing studies are not representative and comparable due to the multi-aetiological nature of non-traumatic SCI. For instance, WHO estimates that non-traumatic SCI comprises approximately 10% of all SCIs, whereas proportions ranging between 30% and 80% of all SCIs have also been suggested (1). The life expectancy of SCI individuals is reduced and is dependent on the age at injury. The risk of premature death of SCI patients is over 5-fold greater than the risk in those without SCI. Mortality is highest in the first year after SCI. Mortality is strongly associated with the severity and level of SCI and with the availability of well-timed and high-quality acute medical care. Secondary complications may cause life-threatening conditions after injury, and their occurrence depends on ongoing health maintenance. In most cases, SCI leads to loss of independency. A caregiver, use of assistive technology to perform daily activities, or both are often needed. Social involvement is significantly decreased due to physical limitations, the negative attitude of the general public towards individuals with SCI, and loss of self-esteem. Clinical depression is observed in 20-30% of patients. Only 12% of individuals with SCI are employed at 1 year after the injury whereas 35% are employed after 20 years (2).

1.2 RELEVANT FUNCTIONAL ANATOMY OF THE SPINAL CORD

The spinal cord and the brain compose the central nervous system (CNS). The spinal cord conveys motor, somatosensory, and visceral information in two directions. Descending tracts carry commands from the brain to the efferent organs and ascending tracts provide the brain with sensory feedback from the periphery. The proprioceptive and tactile sensory feedback modulates motor processes and enables accurate and proper voluntary movements (3).

The spinal cord lies in the vertebral canal formed by 32 vertebrae. It extends from the brainstem at the level of foramen magnum and terminates at the first lumbar vertebra. As the spinal cord is shorter than the vertebral canal, the downstream space from the first lumbar vertebra contains peripheral nerves from the lumbar and sacral spinal segments before they exit the vertebral canal (called the cauda equina). Damage to the cauda equina is considered as a peripheral injury although it lies in the vertebral canal.

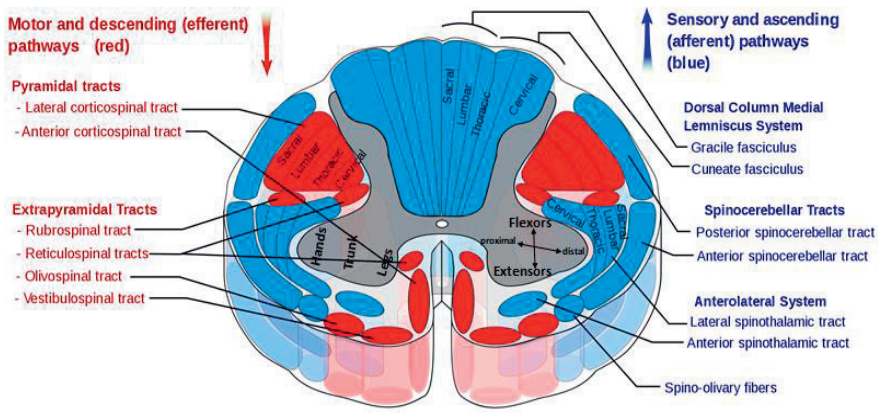

Figure 1 Somatotopic organization of ascending and descending pathways and nuclei of the ventral horn in the cross-sectional spinal cord. Figure is modified from Polarlys and Mikael Häggström. The original picture is licensed under the Creative Commons Attribution-Share Alike 3.0 (https://creativecommons.org/licenses/by-sa/3.0/deed.en).

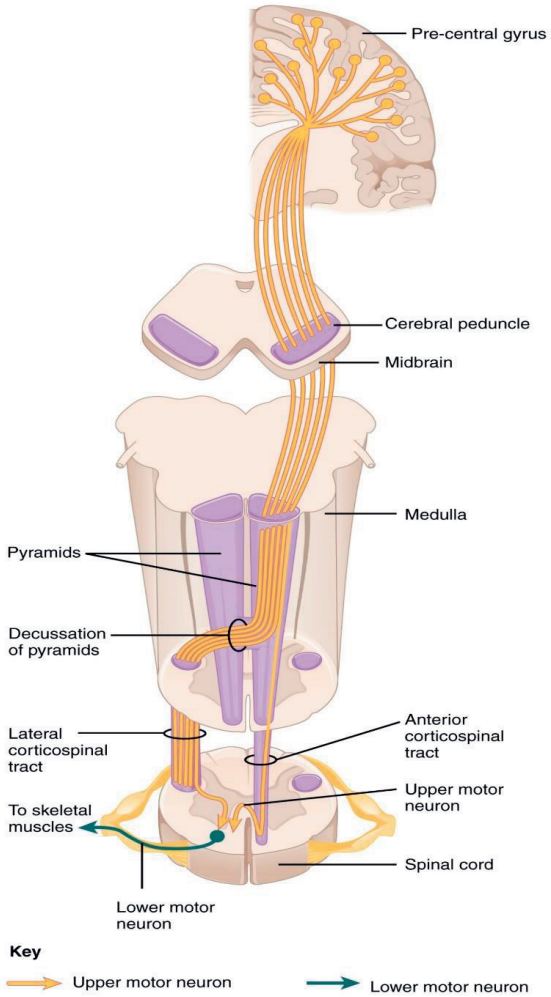

Figure 2 Corticospinal tract. Axons of the upper motoneurons (pyramidal cells) descend from M1 and synapse the lower motoneurons in the spinal cord. Lower motoneuron axons form peripheral nerves that innervate muscles. Reprinted from Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/. The original picture is licensed under the Creative Commons Attribution 3.0 license (https://creativecommons.org/licenses/by/3.0/deed.en).

The grey matter of the spinal cord has a transversal organization and is divided into 30 segments. Each segment gives rise to 30 pairs of peripheral nerves (the right and the left) that innervate a particular part of the body. Tracts and nuclei of the spinal cord are organized in a somatotopic fashion (Figure 1). Based on this knowledge, the character and level of damage to the spinal cord can be defined in a neurological examination (4).

Voluntary movement is the product of complex interactions of different levels of the motor system. It begins from an internal desire to move, possibly generated in the limbic system and in the posterior parietal cortex. Thereafter, planning and programming of the movement are processed in the premotor and supplementary motor cortices (5). Ultimately, the motor output from cortex descends along the corticospinal tract to the muscles (Figure 2). Voluntary motor control is implemented through the corticospinal tract (CST), which is responsible for fine skilled movements in distal limb muscles. CST consists of axons of upper motor neurons in the primary motor (40%), premotor (40%), and somatosensory cortices (30%). In humans, 15-20% of these axons form synapses directly with the lower motor neurons (6). The remaining axons terminate on interneurons in the spinal cord. CST terminations extensively overlap with interneurons of afferent axons that provide feedback on muscle spindle tension and joint position critical for precise movements (3). Finally, the lower motor neurons in the spinal cord activate muscles to execute the movement.

Extrapyramidal, vestibulospinal and rubrospinal tracts provide control of axial and proximal musculature responsible for balance and body posture during movements (7).

1.3 DEFINITION AND CLASSIFICATION OF SCI

SCI interrupts neuronal information flow from the brain to the spinal cord. SCI results in diminished or completely absent function of motor and sensory pathways. Additionally, visceral and autonomic regulation are affected. Neuropathic pain and spasticity are often present in SCI individuals. According to the National Spinal Cord Injury Statistical Center in the United States, most SCI cases (47%) present with incomplete tetraplegia, followed by incomplete and complete paraplegia (20%), and complete tetraplegia (11%) (8). The American Spinal Injury Association (ASIA) developed an internationally recognized impairment scale (ASIA impairment scale, AIS) for assessment and classification of SCI (9). The AIS examination is easy to perform during primary examination in the emergency room and in subsequent regular neurological assessments. However, AIS testing is possible

Table 1 *Scoring of motor function in the American Spinal Injury Association Impairment Scale (AIS) and Manual muscle test (MMT), ROM indicates range of motion.*

Score	Description
0	Total paralysis
1	Palpable or visible contraction
2	Active movement, full ROM with gravity eliminated
3	Active movement, full ROM against gravity
4	Active movement, full ROM against gravity and moderate resistance
5	Normal active movement, full ROM against gravity and full resistance expected from a healthy person
NT	Not testable

for only conscious and cooperative patients as it requires performing tasks on demand. AIS examines sensory and motor function throughout all dermatomes and myotomes and defines the neurological level of SCI and the severity of injury.

AIS assessment is based on the evaluation of functions in myotomes and dermatomes. A myotome consists of a group of muscles innervated by a single motor nerve; similarly, a dermatome is a skin area innervated by a single sensory nerve. A numerical order of myotomes and dermatomes is identified by a numerical order of corresponding spinal segments.

Motor function from 10 spinal segments C5 - T1 and L2 - S1 is assessed bilaterally in the key muscles of the myotome. The scoring of motor function is presented in Table 1. Motor level is defined as the most caudal myotome having antigravity muscle function (score 3/5) on both sides, assuming that upper myotomes have a normal function (5/5).

Sensory function is assessed bilaterally from 28 dermatomes innervated from C2-S5 spinal segments (Table 2). Pin prick and light touch tests assess tactile and pain sensations that transverse along dorsal and anterolateral columns of the spinal cord. The sensory level of SCI is defined as a dermatome with the most caudal normal sensation in tests on both sides, with normal sensation rostrally.

Table 2 *Scoring of sensory function in AIS*

Score	Description
0	Absent
1	Altered (hypaesthesia, hyperesthesia, deviated sensation)
2	Normal as expected from a healthy person
NT	Not testable

Thereafter, the neurological level of injury is determined as the most rostral spinal segment with both intact sensation and antigravity muscle function.

Severity of injury is classified from A to E and defines whether SCI is complete or incomplete (Table 3). Incomplete injury (B-D) is characterized by preserved partial motor or sensory function below the neurological level. In complete injury (A), no motor and sensory function is observed in the lowest sacral segments (S4/S5). Imaging studies add accuracy to the diagnosis and provide information on the extent of injury. The best option for visualization of soft-tissue damage is magnetic resonance imaging (MRI) with T1- or T2-weighted mode. Computer tomography (CT) is the best option to detect bone pathology in traumatic SCI. A final diagnosis is established at the chronic stage (1 year after the injury), when spontaneous recovery is presumed to be complete.

1.4 PATHOGENESIS OF SCI

The mechanisms of injury in traumatic and non-traumatic SCI are different. This is due to differences in time course of the injury and in aetiology. Traumatic SCI results from a sudden event of trauma to the spinal cord that triggers pathophysiological processes consisting of explicit stages. In contrast, non-traumatic SCI develops gradually (from days to years, except in spinal cord infarction) and the underlying disease specifies its pathophysiology.

Four traumatic biomechanisms damage the spinal cord. In SCI, flexion, extension, and axial rotation of the spine and vertebral compression can

Table 3 *AIS Impairment Scale*

Grade	Complete/ Incomplete	Description
A	Complete	No sensory and no motor function at S4/5
B	Sensory incomplete	Sensory function preserved below the neurological level and at S4/5. No motor function below the neurological level
C	Motor incomplete	Motor function is preserved below the neurological level and more than half of the key muscle below the neurological level have grade < 3 (0, 1, 2)
D	Motor incomplete	Motor function is preserved below the neurological level and more than half of key muscles have a grade ≥ 3 (3, 4, 5)
E	Normal	Normal sensory and motor function at all segments

be combined in a single case. The spinal cord may be stretched, compressed, dislocated, or crushed by fracture or by acutely ruptured intervertebral discs. Traumatic SCI results from primary and secondary injuries. The primary injury constitutes an immediate phase that lasts up to 2 hours after traumatic exposure. It is characterized by disruption of spinal cord tissue and vascular changes, including vasodilatation, hyperaemia, and petechial haemorrhages. Secondary injury is characterized by a cascade of biochemical and cellular reactions initiated by the primary injury. It includes an acute phase (up to 2 days), an intermediate phase (days to weeks), and a late phase (weeks to months). The acute phase is characterized by inflammation, oedema, haemorrhages, and changes in myelin and neurons. Recovery from the injury starts during the intermediate phase. This involves astroglial scarring, revascularization, restoration of the blood-brain barrier, and resolution of oedema. Formation of astroglial and mesenchymal scars is finished in the late phase (10). Pathophysiological scenarios of non-traumatic SCI depend on its aetiology. Pathophysiology of infectious myelopathies is characterized by prevalence of cellular toxic damage to the spinal cord. Inflammatory myelopathies are characterized by biochemical and immunological mechanisms. Vascular myelopathies are triggered with tissue ischemia. Neoplastic processes, abscesses, and syringomyelia damage the spinal cord

by compression and resemble to some extent the traumatic compressive SCI. However, the time course of injury is important, as compensatory mechanisms are activated in chronically developing non-traumatic myelopathies. Non-traumatic myelopathy may occur acutely (e.g., in spinal cord infarction) or have a chronic course (e.g., transverse myelitis in multiple sclerosis). Well-recognized signs of developing myelopathy and successful targeted treatment enable a more favourable prognosis for recovery after non-traumatic than traumatic SCI (11).

1.5 CLINICAL REPRESENTATION IN SCI

Damage to the spinal cord is characterised by the level and extent of injury. Damage to the white matter that constitutes the ascending and descending tracts generates an upper motor neuron lesion characterised by muscle weakness, increased muscle tone, and increased tendon reflexes. Damage to the grey matter that contains the cell bodies of the motor neurons generates a lower motor neuron lesion and is characterised by muscle weakness, muscle hypotonia, and reduced or absent tendon reflexes. Characteristics of damage to the spinal cord give rise to a range of neurological dysfunction patterns, which can include a single upper or lower motor neuron lesion (12). However, most SCIs have combined lesions with features of lower motoneuron lesion at the segmental level and upper motoneuron lesion below the neurological level of the injury (13,14). In neurological examination, this combined lesion is easier to detect in injuries to the cervical and the lumbar spinal cord innervating the upper and lower extremities.

The clinical representation of SCI is defined by the location, extent, and pattern of damage. Cervical injury accounts for approximately 50% of traumatic SCIs. Classically, cervical SCI is characterized by tetraplegia with sensory and autonomic dysfunction. Bowel and bladder dysfunction are generated by the upper motor neuron lesion. A neurogenic bladder results in bladder hyperreflexia with detrusor-sphincter dyssynergia. A neurogenic bowel presents with constipation, although sphincter reflexes are spared. Cardiovascular dysfunction is characterized by bradycardia and orthostatic hypotension. A very high cervical lesion at the level of the foramen magnum may be accompanied with signs of lower cranial nerve damage, resulting in dysarthria, dysphagia, and dysphonia. Some non-traumatic causes, such as Arnold-Chiari malformation, syringomyelia, or multiple sclerosis may affect most rostral cervical spinal cord. Cervical injury at C3-C5 elicits a high risk of developing respiratory failure due to lesions in the upper and

lower motoneurons innervating the diaphragm and other muscles essential for respiration (12).

Thoracic injury accounts for about of 35% of traumatic SCI. Typical neurological consequences are paraplegia with sensory deficits. Involvement of the autonomic system depends on the level of the injury. Neurogenic bowel and bladder are caused by upper motor neuron lesions. The sympathetic preganglionic neurons extend through T5-L6 spinal segments. The severity of sympathetic dysfunction depends on the level of injury; it is progressively more severe in SCIs rostral to T6. Supraspinal parasympathetic control is not affected in SCI as it is transmitted by the vagus nerve, which exits the CNS at the medullary level. However, a tendency to hypotension is observed, since parasympathetic vessel control is not balanced with the sympathetic system (12).

Lumbar SCI includes injuries to the conus medullaris and cauda equina. The conus medullaris encompasses 10 spinal segments (L5-S5) within two vertebrae (T12-L1). Separation to segmental damage is not feasible in this area. Lesions of the conus medullaris result in paraparesis with lower motoneuron-type features, sensory deficit, and atonic bladder and anal sphincter. The cauda equina is a bundle of nerves originating from the L2 segment in the spinal canal below the conus medullaris. Damage to the cauda equina leads to similar symptoms and signs as conus medullaris damage (15). Most patients also have severe low back pain. Differentiation of conus medullaris and cauda equina lesions is difficult in clinical examination and neuroimaging is usually needed for diagnosis (12).

Approximately 20% of traumatic and nontraumatic SCI exhibit patterns of neurological dysfunction, suggesting anterior cord, posterior cord, unilateral cord (Brown-Sequard), or central cord syndromes and their combinations. These syndromes appear as incomplete SCI with typical clinical presentations. The remaining 80% have signs of complete and incomplete SCI with random pattern of injury (16).

Severity of SCI is characterised by the extent of injury. International Standards for Neurological Classification of SCI (ISNCSCI) defines complete SCI as the absence of sensorimotor function at the S4-5 segments. A complete SCI with a zone of partial preservation (ZPP) implies that some pathways of CST are spared below the neurological level of injury, contributing to somewhat voluntary control of the corresponding muscles (12).

2. BACKGROUND: UP-TO-DATE SCI REHABILITATION

Current acute management of SCI has improved to the extent that the risks of mortality from secondary conditions have diminished and the life expectancy of individuals with SCI approaches that of the general population. Therefore, rehabilitation of SCI individuals is needed to improve their quality-of-life by enabling social life and work. The CST exhibits plastic changes in response to motor training or injury (17). The CST is a major pathway for voluntary movements and CST lesions strongly correlate with motor deficit; thus the CST is a principal target for motor rehabilitation (18). The CST is accessible to external stimulation that enables application of non-invasive neuromodulation techniques in rehabilitation.

2.1 CONVENTIONAL REHABILITATION

Conventional rehabilitation programs are available in most rehabilitation centres and outpatient clinics. These include occupational and physical therapies. Conventional rehabilitation aims to enhance remaining skills, regain lost functions, and adjust to everyday living by applying compensatory strategies. For tetraplegic patients, the primary goal is to improve hand skills (grooming, eating, dressing, basic manipulation of objects, transfer to wheelchair). For paraplegic patients, the goal is to achieve ambulation. In general, exercising as a daily routine is highly important for individuals with SCI for maintaining motor and cardiopulmonary function and preventing muscle atrophy and vein thrombosis (19).

Restorative therapy in conventional rehabilitation includes exercise training. Physiotherapy aims to increase muscle strength and reduce muscle hypertonia, pain, and spasticity, which disturb training and reduce overall motor performance. Numerous repetitions during motor-task training are expected to induce plastic changes restoring motor function. To regain a lost function, the patient needs to perform the exercises with high motivation in multiple physiotherapy sessions. With the help of an occupational therapist, the regained function should be integrated into the activities of the patient's everyday life for actual functional improvement (20).

Exercise training can include functional electrical stimulation (FES). FES consists of electrical stimulation (ES) of peripheral nerves, muscles, or both and concomitant voluntary effort to execute an artificially induced movement. FES aims to selectively contract the muscles participating in the weak movements requiring improvement. The FES effect is mediated through activation of motor- and sensory-muscle fibres resulting in generation of reflex-based coordinated muscle contractions. A special FES setup may also induce antidromic activation of motor pathways with subsequent depolarization of the motoneurons in the spinal cord. In this case, FES could exert a neuromodulation effect in the spinal circuits (21). FES increases muscle strength and may improve blood circulation, muscle spasticity, muscle atrophy, and range of motions (22).

In general, conventional rehabilitation aids motor recovery after SCI, with better outcomes in less severe cases. For a patient with complete SCI, functionally meaningful restoration of motor function is not possible. Motor recovery may be better when conventional rehabilitation includes FES (23). However, a systematic review evaluating the effectiveness of 22 common physiotherapies of SCI patients justified administration of only four interventions (fitness training, hand and wheelchair training, and FES) with low-power evidence (24). For functional motor recovery leading to patient autonomy, conventional rehabilitation should be supplemented by other approaches.

2.2 NEUROMODULATION TECHNIQUES

The International Neuromodulation Society defines therapeutic neuromodulation as the alteration of nerve activity through targeted delivery of a stimulus to specific neurological sites in the body. The effects of stimulation targeting M1 and the spinal cord and their combinations have been tested in clinical trials.

2.2.1 Spinal cord stimulation

Spinal-cord ES delivered as a tonic subthreshold current facilitates voluntary motor activity below the level of injury in complete and incomplete SCI (25,26). This effect could be mediated by upregulation of propriospinal circuits and enhanced supraspinal input leading to increased excitability of the motoneuronal pool (25). This neuromodulatory effect is also seen in proprioceptive afferents within the dorsal roots expressed as

increased spinal reflexes (27). The spinal cord can be stimulated noninvasively with transcutaneous electrodes.

Epidural stimulation requires surgical implantation of electrodes. Epidural stimulation activates large-diameter sensory afferents that synapse onto interneurons and motoneuronal circuits (28). Epidural stimulation has higher spatial resolution and activates neuronal fibres more selectively than transcutaneous stimulation. Spinal stimulation is often combined with an activity-based rehabilitation program and supports functional recovery by inducing adaptive neuroplasticity (29). In restoration of motor function, research on epidural stimulation is mainly focused on walking rehabilitation.

Epidural stimulation is usually applied to lumbar spinal segments. It targets the central pattern generator (CPG), an intrinsic spinal network capable of generating rhythmic stereotyped walking-like behaviour independently of supraspinal input when triggered by sensory input below the injury (30). Lumbar spinal stimulation is thus an attractive technique for rehabilitation of ambulation after SCI. Some studies have applied epidural stimulation for upper-limb rehabilitation with less promising results (31,32). This may be explained by the absence of a CPG-type intraspinal network in the cervical spinal cord. The cervical spinal cord has a more complex organization of neuronal circuits needed for non-stereotyped sophisticated hand movements. Spinal stimulation research demonstrates the potential for functional motor recovery after SCI. Particularly, a regained voluntary control over lower-limb muscles in complete SCI during stimulation is a promising result. However, data on follow-up evaluation of observed effects relevant for functional recovery are scarce. There is currently no strong evidence on the effectiveness of the intervention due to small sample sizes and lack of proper controls in the studies (33). Additionally, the limitations of epidural stimulation include risk of infection, expensive equipment, and time-intensive rehabilitation. Nonetheless, clinical trials have demonstrated the feasibility and safety of spinal stimulation.

2.2.2 Transcranial stimulation

The role of the M1 in SCI rehabilitation has also been studied. The motor system immediately responds to lesions of the spinal cord by reorganization of M1 (17). This results in reduction of cortical representation areas of weak muscles and expansion of representations of the strong muscles. In cervical myelopathies, mild symptoms are associated with extension of cortical motor representations, whereas patients with severe symptoms display reduced motor representation areas

(34). Spared corticospinal connections of the weak muscles are the most probable substrate for rehabilitation. Strengthening of motor descending activation would reinforce plasticity within the CST and enhance transmission along residual pathways ultimately associated with augmentation of motor output (35).

Transcranial direct current stimulation (tDCS) is used for modification of cortical excitability. tDCS delivers a continuous subthreshold current over the scalp. Anodal tDCS promotes neuroplasticity plausibly through depolarization of intracortical axons and pyramidal neurons, leading to increasing cortical excitability that alters the firing rate of neurons (36). Thus, tDCS therapy may contribute to neuroplasticity within the cortex and along corticospinal projections. A meta-analysis of randomized sham-controlled blinded clinical trials in SCI indicated efficacy of anodal tDCS in functional recovery with a small effect size. However, there was no significant difference in muscle strength between active and sham tDCS (37).

In transcranial magnetic stimulation (TMS), a rapidly changing electric current is delivered to a stimulation coil. This current generates a strong magnetic field. The magnetic field non-invasively induces an electric field (EF), which induces a secondary current in the brain. TMS applied over the M1 at the motor threshold (MT) activates pyramidal cells trans-synaptically via intracortical neurons (38). The TMS-induced neuronal output from M1 is recorded as a motor-evoked potential (MEP) from the muscles innervated from the stimulated M1 area.

Repetitive TMS (rTMS) represents a sequence of high- ($\geq$ 1 Hz) or low- ($<$ 1 Hz) frequency or patterned pulses and can modulate cortical excitability. rTMS evokes action potentials in cortical neurons and may enhance synaptic transmission of intracortical connections to pyramidal cells leading to relevant neurophysiological changes in CST (39). A stimulation session consisting of hundreds of TMS pulses could induce plastic changes within the residual CST and contribute to functional recovery. Despite extensive investigation of the effects of rTMS, only a few clinical trials have been conducted in SCI individuals. Application of multi-session rTMS can induce some functional improvement in SCI individuals (35). Overall, the available data are inconsistent and likely depend on the parameters of the rTMS protocol and severity and level of SCI (35). Thus, it is difficult to estimate the effectiveness of rTMS in patients with SCI.

2.3 INDUCING NEUROREGENERATION IN SCI

Information on the cellular and molecular mechanisms underlying SCI has accumulated during the last three decades. Promising preclinical animal experiments have led to initial phases of clinical studies.

SCI results in damage to the spinal cord parenchyma with disruption of ascending, descending, and intraspinal connections. In subacute and chronic stages of SCI, restoration of lost functions is possible if remaining connections compensate for lost connections through axonal sprouting and if injured axons regenerate to form new connections. CNS neurons were considered unable to regenerate until Aguayo demonstrated in 1981 that transected CNS axons can regrow into a transplanted peripheral nerve (40). This discovery emphasized the importance of extrinsic factors of the neuronal environment on regeneration. Fibroglial scar formation within and around the injury epicentre and a range of inhibitory molecules generate an environment inhibiting axonal outgrowth. Several animal studies counteracting this inhibitory environment have demonstrated the safety and potential efficacy for axonal regeneration after SCI and have justified translation of the research to clinical studies (41). The drug cethrin, which modulates responses to proteoglycan (CSPGs), the extracellular molecule of the glial scar, can induce moderate neurological recovery in acute complete SCI patients. However, the small number of the studied patients did not enable conclusions of drug efficacy (42). Another phase I clinical trial studied the effectiveness of intrathecal anti-Nogo-A antibodies against a myelin-associated inhibitor in 52 acute complete SCI patients. Although the results demonstrated limited efficacy, mild adverse effects favoured its administration in acute and subacute SCIs (43). The role of neurotrophic factors, for example brain-derived neurotrophic factor (BDNF), has been extensively investigated in animal studies. BDNF has shown efficacy in prevention of corticospinal neuron death in a spinal-cord injury model (44). BDNF enhances regeneration of injured axons and promotes synaptic strength and collateral sprouting of spared connections. This would be relevant to functional spontaneous recovery in patients with incomplete SCI. However, to provide axonal outgrowth beyond the lesion site and prevent the development of spasticity, high doses, precise localized delivery, and transient administration of BDNF should be considered (45).

Axonal cytoskeletal dynamics, axonal transport, and epigenetic and transcriptional regulation define intrinsic regenerative mechanisms. Although several studies have demonstrated pro-regenerative activity in vitro and in vivo, sufficient knowledge has not accumulated on these mechanisms for clinical trials.

2.4 CELL THERAPY IN SCI

Cell transplantation may benefit from approaches targeting intrinsic and extrinsic factors of axonal regeneration in severe SCIs with extended lesions. Ideally, cell therapy should provide a permissive environment for axonal outgrowth, enhance remyelination, and replace lost neuronal substrate. Mesenchymal stem cells (MSCs), isolated from the bone marrow, can differentiate to neurons. Therapy with MSCs has been extensively studied in animal models of SCI and has restored neuronal tissue integrity leading to functional improvement through anti-inflammatory, neuroprotective, and pro-regenerative activity. While several clinical trials have demonstrated safety of MSCs, poor neurological recovery was observed in chronic SCI patients. The small number of studied patients precludes conclusions of clinical efficacy (47-50). Schwann cells have a principal role in regeneration of peripheral nerves and have been investigated for possible CNS regeneration. To date, three clinical trials on Schwann-cell transplants have been completed. These trials have shown weak neurological recovery and no transplantation-related adverse effects (51-53).

In recent few decades, advances in knowledge on the pathophysiology of CNS injury has led to progressive growth in neuroregenerative medicine. The complex nature of post-injury processes in the spinal cord established several directions in research to investigate different potential therapeutical approaches. An extensive body of preclinical data on pharmacological and cell therapies have demonstrated safety and potential neurological recovery in SCI models. However, despite several clinical trials, a major breakthrough in regenerative medicine has not yet appeared. Nevertheless, substantial knowledge has been obtained that has revealed novel concepts and important pathways towards a SCI cure. It is becoming clear that single treatments cannot fully restore function after severe SCI, which involves a complex interplay of intrinsic and extrinsic factors after injury. Rather, combinatory approaches may be able to provide meaningful recovery (46).

3. PAIRED ASSOCIATIVE STIMULATION

3.1 INTRODUCTION TO THE METHOD

In paired associative stimulation (PAS), two stimulations are applied at different sites of a neuronal circuit with convergence at yet another site. PAS, as introduced by Stefan and Classen in 2000 (54), combined simultaneous TMS and peripheral nerve stimulation (PNS). Donald Hebb postulated in 1949 that if the presynaptic cell and its postsynaptic target activate synchronously and persistently then the synaptic connection between them becomes stronger (55). The development of the PAS protocol was inspired by the model of associative long-term potentiation (LTP) in animal studies, which were based on Hebbian synaptic plasticity.

In the original protocol of Stefan and Classen, TMS delivered to the contralateral M1 was timed to converge on pyramidal cells simultaneously with ascending somatosensory input induced by median nerve (MN) stimulation. The protocol with 90 paired stimuli led to facilitation of corticospinal transmission, demonstrated as an increase of MEP amplitudes. This variant of the PAS protocol is called cortical PAS, as it is designed to induce synaptic changes at the cortex.

PAS can induce bidirectional changes in the strength of targeted synapses depending on time relationship of the induced neuronal activities. Stefan et al (2000) showed that a PAS protocol with nearly synchronous neuronal inputs converging at M1 yielded MEP facilitation; in contrast, a PAS protocol that separated the cortical neuronal activations induced MEP suppression. The observed effects evolved rapidly, remained persistent but were reversible, and had a specific topography. These properties of PAS effect suggested that the PAS mechanism parallels the associative long-term plasticity. Additionally, cellular mechanisms of PAS were shown to be similar to those for LTP induction supported by pharmacological studies where PAS-induced changes revealed dependence on NMDA receptors and calcium-channel transport (56,57).

The properties and potential of PAS have been studied extensively (56). In addition to plastic changes in M1 induced by the original PAS protocol, several PAS modifications have displayed after-PAS LTP/LTD-like plasticity in M1 when TMS to M1 was coupled with afferent visual, auditory, nociceptive, or proprioceptive stimuli and with stimulation of the cerebellum, basal ganglia nuclei, the supplementary motor area, and the posterior parietal cortex. PAS protocols targeting specifically the primary

sensory cortex and the spinal cord have also been designed (56). Thus, PAS allows investigation of synaptic efficacy in neuronal circuits in healthy individuals and in individuals with different neurological conditions (56,58).

The effects of even a highly focal single stimulation are not limited to the site of stimulation. The induced effects spread extensively throughout interconnected neuronal circuits in the brain. PAS has the potential to be more beneficial than unpaired stimulation, since converging activations induced with a two-site stimulation can narrow the induced effect to specific networks in CNS. This precision of PAS enables investigation of neuronal populations of scientific interest or therapeutic effects on selective targets.

3.2 CELLULAR MECHANISMS OF PAS

PAS-induced synaptic plasticity is plausibly mediated by LTP- or long-term depression (LTD)-like mechanisms. LTP was tested early in in vitro experiments on hippocampal slices, where patterned ES to presynaptic axons led to elevation of excitatory postsynaptic potential recorded from a postsynaptic cell (59). According to the "classical" LTP theory, the effect starts when induced neuronal activity triggers a growth of calcium concentration in the postsynaptic cell. Thereafter, a calcium-dependent second messenger system activates protein phosphorylation and initiates the early stage of long-term synaptic plasticity. When such neuronal activation is maintained, alteration in protein gene transcription in the postsynaptic cell leads to growth of new spines in the synapse and brings about long-lasting synaptic changes. The level of intracellular calcium plays a crucial role in determining the polarity of long-term synaptic changes. A strong depolarization of the postsynaptic membrane enables a rapid and high elevation of intracellular calcium concentration resulting in LTP; in contrast, a weak depolarization mediates slow and insufficient intracellular calcium concentration resulting in LTD. As an endpoint of this cellular cascade, when LTP is induced, new glutamate AMPA receptors are inserted from a vesicular pool of a postsynaptic cell that increases its sensitivity to the neurotransmitter glutamate and makes the synaptic transmission more efficient. In contrast, during LTD induction, a removal of AMPA receptors leads to weakening of the synapse. Associative long-term plasticity occurs when an input to a postsynaptic cell is synchronized with postsynaptic depolarization, replicating a natural course of neuronal transmission. In this case, the mechanism of long-term plasticity induction can be explained

with spike-timing-dependent plasticity (STDP). STDP is a biological process that adjusts strength in synaptic connections in the CNS (59).

3.3 PAS FOR MOTOR REHABILITATION

Plasticity induces structural and functional changes within the target circuits and systems (60). Plasticity is the basis for learning, memory, acquisition of motor and cognitive skills, and adaptation to injury. Animal models have demonstrated the possibility for long-term synaptic plasticity to be induced with conditioned stimulation in experimental settings (61). PAS represents a stimulation paradigm to modulate synaptic efficacy in a long-term manner in humans. After-injury residual connections are the principal target for PAS (62). A single PAS session results in a transient increase of MEP amplitudes (58). Importantly, this neurophysiological change is supported by an increase in behavioural output in healthy and SCI individuals and is thereby relevant for motor restoration (63,64). Most PAS experiments have studied the efficacy of a single PAS session. A 4-week PAS improved leg muscle function in some stroke patients (65). Physiologically relevant outcomes of long-term PAS make this approach attractive for motor rehabilitation in CST lesions.

3.4 COMPONENTS OF A PAS PROTOCOL

Understanding the mechanisms of PAS enables the development of PAS protocols that operate in different CNS circuits. PAS consists of TMS and PNS. TMS and PNS parameters such as timing, intensity, and pattern of stimuli are considered when designing a PAS protocol for investigation of properties of the nervous system and for achieving desirable therapeutic effects.

3.4.1 TMS in PAS

Depending on the type of the TMS coil and stimulation intensity, TMS has a spatial resolution of approximately 0.5-1 cm and a limited depth of stimulation up to 4 cm (66). A TMS navigation system ensures accurate delivery of TMS pulses to a chosen spot in M1 within one stimulation session and between sessions (Figure 3 and 5). TMS is painless and does not have serious adverse effects when delivered with a single pulse at low frequency (67).

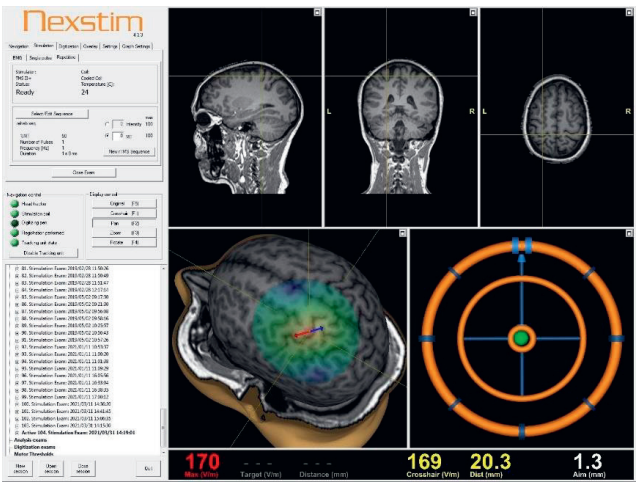

Figure 3 *Navigation screen in eXimia NBS software. The upper row displays sections of the subject's MR image. In the lower left corner of the NBS display with the subject's MRI, a red-blue arrow corresponds to the TMS coil placed over the subject's head. The centre of the coil is placed over a spot in M1 for ADM. Navigation of the coil is performed with the TMS navigation system. In the lower right corner of the NBS display, the Aiming Tool guides the position of the coil exactly to the chosen spot. The correct coil position indicator (green dot) considers the location and rotation angle of the coil.*

3.4.2 PNS in PAS

Transcutaneous peripheral electrical nerve stimulation induces a depolarization in the nerve underlying the stimulating electrodes. This neuronal activity distributes along the fibres of the nerve in opposite directions, orthodromically, and antidromically. At a sufficiently high stimulus intensity, the antidromic impulse travels along the motor fibres up to the spinal cord. As the antidromic impulse reaches cell bodies of the motor neuron pool in the anterior horn, a small portion of these alpha motor neurons backfires and elicits orthodromic transmission towards the innervated muscle fibres, which is recorded as a F-response (68, 69). The presence of F-response in deafferented animal and human models indicates that F-response requires direct activation of motor axons and does not involve sensory reflex arch (70). Thus, a F-response reflects a peripheral conduction time along CST and activation of lower motor

neuron pool, providing the parameters of our PAS protocol. The values of an individual F-response intensity and latency are used for setting PNS intensity and an ISI in a PAS protocol, respectively.

Visible muscle contraction during a nerve-conduction study indicated orthodromic activation of motor fibres and is observed as a M-response in EMG. Importantly, the M-response is elicited with lower intensity than the F-response, which is evoked by an electrical stimulus at supramaximal intensity.

In addition to motor fibre activation, PNS applied to the mixed nerves also elicits orthodromic activation along the sensory fibres. This activation travels up to the dorsal root ganglion.

3.4.3 Interstimulus interval

Unlike the original cortical PAS, a spinal PAS protocol is adjusted to coincide the descending and the ascending volleys at the level of the spinal cord within the CST (64,71,72). Such a PAS protocol supposedly facilitates corticospinal transmission by means of strengthening the corticomotoneuronal synapses. ISI defines the time relationship of delivery of external stimuli in the PAS protocol. Consequently, ISI defines the time of arrival of the induced pulses at the targeted synapse.

PAS-induced long-term plasticity is governed by principles of STDP, which define the effective time windows for synaptic changes. In a cell model, depolarization of the presynaptic membrane within 20 ms before a postsynaptic activation induces LTP at the synapse. If a presynaptic membrane is activated within 100 ms after postsynaptic depolarization, LTD will be induced. When the neuronal activations at the synapse are separated by 120 ms or more, such interaction is not relevant for synaptic strength change (73). Notably, the magnitude of synaptic change is higher when pre- and postsynaptic activations occur closer in time (74). In humans, greater MEP potentiation occurred when converging PAS pulses were nearly synchronised. Even a small shift in time of the pulse interaction induced MEP depression or no effect (64,75).

In studies employing the original PAS protocol with MN stimulation, an ISI of 25 ms was set based on an established standard value of the N20 latency of MN somatosensory-evoked potential in a healthy population (54,76). For targeting the corticomotoneuronal synapses in spinal PAS protocol, ISI calculation can be performed using MEP, C-root (the peripheral conduction time along the motor nerve measured from the cervical spinal root to the muscle), and F-response latencies (64,77).

3.5 PAS PROTOCOL ISSUES IN SCI

3.5.1 Determination of ISI

SCI initiates structural and functional plasticity in the spinal cord and supraspinal level as a part of natural recovery. This reorganization can be observed in electrophysiological and imaging studies. MEPs, which index functional integrity of CST, are often modified in individuals with SCI. MEP latencies in arm and leg muscles can be delayed by 2-15 ms, most likely due to post-injury demyelination and loss of large diameter corticospinal axons (78). Thus, a PAS protocol utilizing an ISI based on an average value of neurophysiological measures is not appropriate for SCI individuals and an individual calculation of ISI is required. However, even individual measurements may be complicated with spasticity, which hampers interpretation of MEPs and F-responses. In particular, significant spasticity blurs the exact onset of MEP and F-responses due to spike-contaminated baseline. This compromises application of traditional PAS in SCI patients. A high-frequency PNS train was applied in the present PAS protocol to avoid such problems. A high-frequency PNS train consisting of 6 pulses could significantly prolong postsynaptic depolarization (by 50-100 ms) and increase the probability of pulse coincidence when precise determination of ISI is impossible.

3.5.2 Significance of PNS intensity

PNS must be delivered at an intensity sufficient to ensure antidromic activation reaching spinal motoneurons. In the present PAS protocol, PNS intensity is therefore set at an individual F-response intensity that is a direct equivalent of antidromic motoneuronal depolarization needed for PAS actualization. If PNS intensity is insufficient to activate motoneurons, spinal PAS fails to induce neuroplasticity (79,80).

3.5.3 High-intensity TMS

Individuals with SCI undergo cortical reorganization due to plasticity related to sensorimotor changes. Reshaped cortical maps can be expressed with reduction or expansion into other cortical muscle representations (81). PAS targets a specific pathway corresponding to a particular muscle. An optimal site of the specific muscle activation, a hotspot, is usually defined for TMS. In some SCI patients, the reshaped cortical maps prevent reliable

detection of such a hotspot of the target muscle. Instead, inconsistent, low-amplitude MEPs can be elicited from several suboptimal cortical sites. In the present PAS protocol, TMS is administered at maximum stimulator output (MSO). A high-intensity TMS activates a large area in M1 and thus increases the probability of involving a cortical site with the optimal cortical representation of the target muscle. Furthermore, high-intensity TMS evokes multiple descending impulses corresponding to direct (D-waves) and indirect (I-waves) activations of the pyramidal cells (38). These multiple descending activations result in a greater chance for pulse coincidence in the corticomotoneuronal synapses, thus increasing PAS efficacy (47).

4. AIMS OF THE DISSERTATION

The studies of this dissertation sought to explore the therapeutic potential of a new approach for rehabilitation of motor function in individuals with SCI. For this purpose, a modified PAS protocol, which was expected to be more robust than classical PAS setup in the injured CST, was applied for SCI patients.

The aims of the healthy-subject studies (studies I, II, III) were as follows:

1. to identify the most effective settings of the PAS protocol for inducing stronger and longer-lasting potentiation of CST:
   a. experiment 2.1 and experiment 3.2 sought to define the optimal frequency of a PNS train in the PAS protocol
   b. experiment 3.1 searched for the optimal frequency of pairings in the PAS protocol
   c. experiment 3.3 tested the PAS protocol with a paired-pulse TMS

2. to test our modified PAS regarding feasibility in SCI patients,
   a. study I assessed the PAS protocol in a range of ISIs, addressing possible inaccuracy in ISI determination in SCI individuals
   b. experiment 2.2 assessed the PAS protocol with TMS delivered to the suboptimal target in M1, addressing possible inaccuracy in M1 mapping in SCI individuals

The aims of the patient studies (studies IV, V, VI) were as follows:

1. to investigate efficacy, sustainability of the result, and safety of long-term PAS in chronic SCI individuals:
   a. in individuals with SCI of traumatic origin in studies IV and V
   b. in individuals with SCI of different non-traumatic origins.

2. to compare the efficacy of PAS to PNS only in studies IV and V.

3. to investigate efficacy of intervention at different PAS settings derived from healthy-subject studies:
   a. PAS with 50-Hz PNS was employed in studies IV and V
   b. PAS with 100-Hz PNS was employed in study VI

5. MATERIALS AND METHODS

5.1 TRANSCRANIAL MAGNETIC STIMULATION

TMS was executed with an eXimia magnetic stimulator (Nexstim Ltd., Helsinki, Finland). The device houses a figure-of-8-coil with an outer loop diameter of 70 mm generating biphasic waveform pulses (length 230 μs). The figure-of-8 coil provided a high-resolution M1 mapping, which is important for targeting specific neuronal pathways in the PAS protocols. eXimia has a Navigated Brain Stimulation (NBS) system (software version 4.3) that utilizes the subject's magnetic resonance images (MRI) of the brain and enables monitoring the coil position with respect to the brain. Individual structural T1-weighted MRIs were obtained for each participant with a 3T Siemens Verio scanner (Siemens Healthcare, Germany).

EMG was recorded with surface self-adhering electrodes attached over the muscles (sampling rate 3 kHz, band-pass filter 10-500 Hz). The EMG device is built into the Nexstim TMS and enables detection and analysis of MEPs in the Nexstim software. MEP size was taken as a peak-to-peak MEP amplitude.

For M1 mapping, TMS pulses were delivered to the foot or hand areas in M1 to find a hotspot of a target muscle. Detection of MEPs was not always feasible in the patients (81). These patients were asked to activate the target muscle to decrease the motor threshold (MT) to facilitate MEPs. Thereafter, the resting motor threshold (RMT) was determined for the target muscle. RMT is the lowest TMS intensity eliciting at least 5 MEPs with an amplitude exceeding 50 μV out of 10 consecutive stimuli. Thereafter, MEP latency was calculated from an average of 10 MEPs elicited by TMS at an intensity of 120% RMT. For patient studies, the target muscles for M1 mapping were abductor digiti minimi (ADM), abductor pollicis brevis (APB), and brachioradialis (BR). For healthy subject studies, the abductor hallucis (AH) muscle was used. TMS was delivered to a hotspot of the target muscle during PAS and MEP measurements.

5.2 PERIPHERAL ELECTRICAL STIMULATION AND F-RESPONSES

A Dantec Keypoint electromyography device (Natus Medical Inc., Pleasanton, CA, USA) and surface self-adhesive silver electrodes with a size of

22 X 30 mm (Neuroline 720, AMBU A/S, Ballerup, Denmark) were used for F-response and MEP measurements and PNS.

In studies on healthy subjects, the right tibial nerve (TN) was stimulated with electrodes placed under the right medial malleolus. EMG was recorded with electrodes placed over the right AH.

In patient studies, the MN, ulnar (UN), and radial nerves (RN) were stimulated. Two electrodes were placed in the middle of the inner wrist and at the sulcus ulnaris antebrachia for MN and UN stimulation, respectively, and above the elbow for RN stimulation. Recording electrodes were placed over the bulks of APB, ADM, and BR muscles. During RN stimulation, stimulating electrodes were gently pressed against the skin to elicit F-responses; the same procedure was done during PAS.

The same electrode position was used for a given muscle during recording of MEPs and F-responses and stimulation for PNS during PAS and PNS for F-response measurements.

A single 0.2-ms square-wave pulse was applied at suprathreshold intensity to measure F-responses. A minimum latency out of 10 F-responses was used for calculation of ISI. For further analysis, maximum F-response amplitude and persistence were also measured.

For determination of PNS intensity in the PAS protocol, a minimum intensity value of F-response elicited with a single 1-ms square-wave pulse was recorded. The F-response intensity was measured with the same pulse duration as PNS given during PAS. For individuals experiencing unpleasant sensations during PNS, EMLA cream (lidocaine 2.5% and prilocaine 2.5%) was applied over the area of stimulation.

5.3 SETTING A PAS PROTOCOL

The PAS protocol was individually adjusted for each healthy subject and patient to increase its efficacy. ISI was calculated by the formula F-latency minus MEP-latency (72) (Figure 4). ISI indicated a delay between TMS and PNS, providing a simultaneous arrival of orthodromic and antidromic volleys induced by a single TMS and the first pulse of a PNS train to the spinal cord (except in study II, experiment 1). A positive ISI standed for PNS preceding TMS and a negative ISI (in a reverse order). Presentation® software (Neurobehavioral Systems Inc., Albany, NY, USA) delivered the trigger pulses for TMS and PNS over PAS according to the calculated ISI. A single TMS pulse was used, except in experiment 3.3. TMS was administered at 100% MSO and

at 96% of MSO for experiment 3.3. PNS during PAS was delivered as a train of 6 pulses in all experiments. The setup of PAS is displayed in Figure 5.

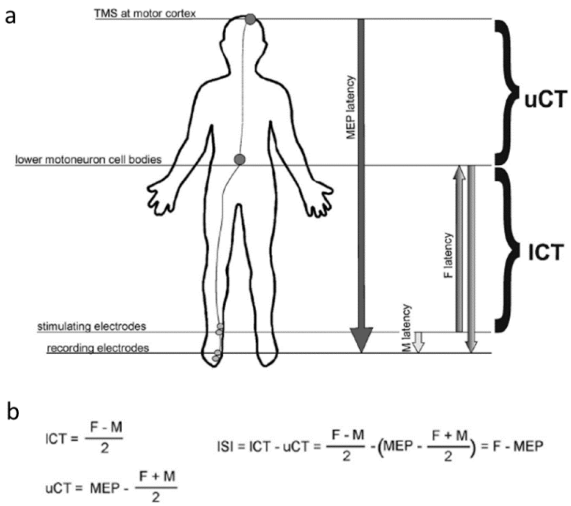

Figure 4 *An example of ISI calculation for lower limb. (a) Schematic representation of the conduction time measurements performed before the PAS protocol. TN stimulation electrode placing is shown. The recording electrodes are placed over AH. uCT, upper motoneuron conduction time; ICT, lower motoneuron conduction time. Schematic representation. (b) Calculation of the individual ISI for the PAS protocol on the basis of measurements shown in (a). F, F-latency; M, M-latency; MEP, MEP-latency from cortical TMS. Modified from Shulga et al, 2015* (72). *The original publication is distributed under Creative Commons Attribution.*

5.4 EXPERIMENTAL DESIGN

For both healthy subjects and patients, the general exclusion criteria were contraindications to TMS. Subjects with epilepsy, metal inclusion in the head area, pacemaker, hearing device, and high intracranial pressure were excluded (82). Additionally, MRI of the brain was performed to exclude subjects with pathological findings. All studies were approved by the ethical committee of Helsinki University Hospital (HUS/1280/2016). The purpose and main principles of the study were described to the subjects and patients. Thereafter, subjects and patients signed an informed consent. They were also informed

that they could discontinue a study at any point without providing a reason. PAS to the one target muscle consisted of 240 TMS-PNS pairings at 0.2 Hz (20 min) except for experiment 3.3.

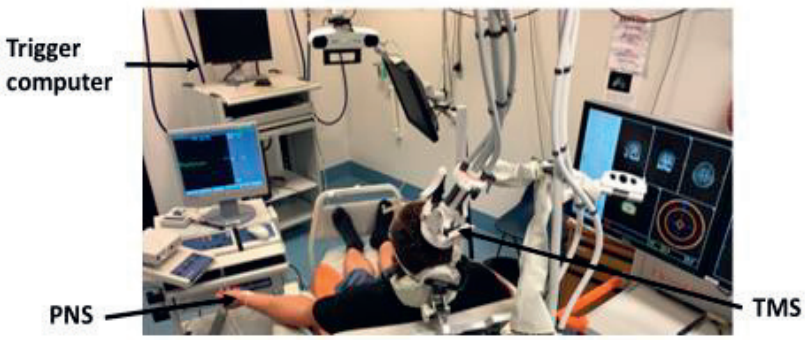

Figure 5 Setup of PAS. The subject is receiving PNS to left MN and TMS to the right hemisphere (cortical representation of left APB). The trigger computer launches the TMS and PNS accordingly to a calculated ISI. Adapted from Tolmacheva et al, 2019 (study II). The original publication is distributed under Creative Commons Attribution 4.0 International License.

5.4.1 Healthy subject studies

All subjects were asked to avoid caffeine intake for at least 6 hours before the stimulation session and to avoid intensive physical activity 1 day before the stimulation. At least 7 days separated the experiments to exclude extension of PAS effect of a previous stimulation session to a subsequent one. All stimulation sessions within one study were performed in a random order. The subjects were asked to imagine movement that would be induced during the stimulation (toe flexion of their right foot) to fulfil the same condition as in our patient study. Detailed information on experimental design of studies I, II, and III is presented in Table 4.

5.4.1.1 STUDY I

Study I addressed the role of an ISI in the PAS protocol. Five subjects underwent 10 stimulation sessions. One PAS session was set with ISI of 0 ms assuming that a TMS pulse is timed to coincide with the first pulse of the PNS train at the corticomotoneuronal synapse. Five PAS sessions were performed,

with ISIs of -10 ms, -5 ms, +5 ms, +10 ms, and +300 ms. A positive ISI indicates that the first pulse of the PNS-induced volley precedes the TMS-induced volley at the corticomotoneuronal synapses before, and a negative ISI indicates that PNS-induced volleys follow the TMS-induced volleys. Control sessions assessed the role of only TMS and PNS applied with the parameters used in the PAS protocol. Additionally, we conducted one PAS session with ISI 0 ms without motor imagery and one PAS with outcome evaluation up to 60 min (Table 4).

5.4.1.2 STUDY II

The aim of the experiment 2.1 was to investigate how the frequency of PNS trains in a PAS protocol affects PAS outcome. PAS protocols with PNS of 25 Hz (PAS/25) and 100 Hz (PAS/100) were compared with the previously used 50 Hz (PAS/50). A control session of PNS only at the most beneficial frequency (100 Hz) derived from this experiment was conducted in 5 subjects.

Experiment 2.2 studied the effect of precision of M1 mapping on PAS. The most effective PAS settings obtained from experiment 2.1 (PAS/100) were applied. After finding a hotspot, four adjacent suboptimal spots in M1 for the right abductor hallucis (AH) were selected equidistant from the hotspot (Study II, Figure 1, B). Thirty MEPs were elicited from these four suboptimal spots. The "weakest" suboptimal spot, determined as a spot with the smallest MEP average, was chosen as a suboptimal TMS target in PAS. PAS was administered for three consecutive days, as repeated PAS produces more persistent effects than a single-session PAS (76). The outcome was measured with 30 MEPs elicited from the stimulated suboptimal spot and the adjacent area, including the hotspot and another suboptimal spot (n=2 x 30 =60 MEPs) immediately after each PAS session and on the eighth day.

5.4.1.3 STUDY III

Experiment 3.1 was designed to investigate possibilities to shorten the PAS protocol without attenuation of its effect. Our previously used PAS/100 at 0.2 Hz (TMS and PNS paired every 5 s, total 240 pairings, stimulation for 20 min) was compared with PAS/100 at 0.4 Hz (TMS and PNS paired every 2.5 s, total 240 pairings, stimulation for 10 min).

Experiment 3.2 investigated whether further increase of PNS frequency in the PAS protocol could strengthen PAS effect. The PAS protocol with a PNS of 200 Hz (PAS/200) and 400 Hz (PAS/400) was compared with a previously applied PAS/100. The objective of experiment 3.3 was to amplify PAS outcome

Table 4 Summary table of healthy subject studies. * except experiment 3.3 where 0,4 Hz PAS tested with paired-pulse TMS at 96% SO and the same rest settings

	Study I	Study II		Study II		
	Experiment 1	Experiment 2.1	Experiment 2.2	Experiment 3.1	Experiment 3.2	Experiment 3.3
Aim of the experiment	Assessment of effectiveness of PAS protocols with different ISIs	Assessment of effectiveness of PAS protocols with PNS train of different frequency	Assessment of effectiveness of PAS protocol with TMS delivered to a suboptimal M1 spot of a target muscle	Assessment of effectiveness of PAS protocols with further increasing of PNS frequency	Assessment of effectiveness of PAS protocol with paired pulse TMS against original protocol with single TMS	Assessment of effectiveness of PAS protocol with shortened stimulation time
Number of subjects	5 (3 males; age 30-60, mean age 40)	10 (5 males, age range 23-40, mean age 32)	5 (3 males, age range 25-61, mean age 38)	9 (3 males, age range 22-42, mean age 32)	10 (5 males, age range 22-46, mean age 37)	5 (2 males, age range 30-39, mean age 34)
General for a PAS protocol	0.2 Hz PAS*: single TMS at 100% MSO*, PNS train of 6 pulses at F-response intensity. Stimulation target- the right AH. Total pulses 240.					
Tested protocols	10 protocols: PAS with ISI ±5 ms, ±10 ms, +300 ms, 0 ms; TMS only; PNS only; ISI 0 ms with evaluation up to 60 min; ISI 0 ms without motor imagery (all other protocols with motor imagery)	5 protocols: PAS/25, PAS/50, PAS/100, TMS only, PNS only	PAS with TMS administered to a suboptimal spot for right AH for 3 consecutive days	3 protocols: PAS/100, PAS/200, PAS/400	PAS with paired pulse 20-Hz TMS at 96% SO	2 protocols: 0,2 Hz PAS (for 20 min), 0,4 Hz PAS (for 10 min)
Evaluation	10 MEPs at 120% RMT before and after the stimulation; in one PAS with ISI 0 ms additional evaluations at 30 and 60 min after the stimulation	30 MEPs at 120% RMT before, after, at 30, and 60 min after the stimulation	30 MEPs measured from a suboptimal spot and an adjacent area before and after the stimulation and at the eighth day	30 MEPs at 120% RMT before, after, at 30, and 60 min after the stimulation		

by adding a second pulse to TMS. A 20-Hz paired pulse TMS was used for pairing the first and the second TMS pulses with the first and the last pulses of a 100-Hz PNS train. The PAS protocol with 20-Hz paired-pulse TMS at the most effective parameters as was derived from previous experiments (0.2 PAS with 100-Hz PNS) was examined.

5.4.2 Patient studies

Long-term PAS with different settings in patients with different SCI aetiology and for different durations were applied in studies IV, V, and VI (Table 5). The studies were registered at clinicaltrials.gov (NCT03459885). Most patients had chronic SCI and tetraplegia. These patients received PAS for hand muscles innervated by MN, UN, and RN. One patient with paraplegia participated in study IV; she received PAS for leg muscles innervated by TN and by peroneal nerves (PN). Nerves supplying the weak muscles were selected for stimulation. During stimulation, patients were asked to imagine induced movement, as motor imagination facilitates corticospinal transmission (83,84). PAS was delivered 5 times a week during the first 2 weeks and 3 times a week thereafter. It was assumed that more intensive exposure in the beginning of the intervention is required when corticospinal connections are still weak. The patients maintained the same conventional rehabilitation and medication during PAS as before the study to ensure that the observed changes were due to PAS. A hand motor score was evaluated before, immediately after PAS, and during the follow up. Additionally, spasticity, sensory function, F-responses, neuropathic pain, functional tests, and hand dynamometry were examined. Patients were not evaluated on the day when they had unusually elevated spasticity, pain, or both in tested extremities to avoid downgrading of the test.

5.4.2.1 STUDY IV

Study IV was an open-label proof-of-principle study of two traumatic SCI patients, one with paraplegia and the other with tetraplegia. The paraplegic patient received PAS to left PN and left TN. Each nerve was stimulated for 30 min during the first 9 weeks; from the week 10 onwards, PAS was administered bilaterally until week 21 of the intervention. The tetraplegic patient received PAS to one hand and an additional control PNS/50 to the contralateral hand within one stimulation session for 12 weeks; during the next 12 weeks PAS was administered to the contralateral hand alone. The patients and evaluating physiotherapist were familiar with the stimulating protocol. Outcome was evaluated during the intervention, immediately after the PAS series, and at a

Table 5 *Summary table of patient studies. F, female; M, male; L/C, lumbar/cervical spinal level.*

	N, patient	Age, sex	Aetiology	AIS	Neurological level	Time after SCI	PAS protocol	Evaluation
Study IV (pilot study)	1	31, F	traumatic	C	L1	1 y	PAS/50 for 12 weeks to TN and PN of each leg	MMT, sensory score, spasticity score, EMG, MEPs, neuropathic pain score before, after, at 1 month
	2	53, M	traumatic	C	C3	1 y	PAS/50 to right MN, UN, RN, and PNS/50 only to left MN for 12 weeks. PAS/50 to left MN, UN, RN next 12 weeks	
Study V	1	38, M	traumatic	B	C7	4 y	PAS/50 to one hand, sham-TMS and PNS/50 to the contralateral hand for 4 weeks	MMT, sensory score, spasticity, neuropathic pain score before, after, at 1 month; F-responses before and after
	2	38, M	traumatic	D	C7	5 y		
	3	42, M	traumatic	C	C4	6 y		
	4	53, M	traumatic	C	C3	3 y		
	5	68, F	traumatic	C	C5	1 y		
		4 males age 38-68, mean 48						
Study VI	1	49, M	epidural abscess at C2-T2	D	C3	1 y	PAS/100 to the weaker hand for 6 weeks, the contralateral hand did not receive stimulation	MMT, spasticity score, mechanical pinch and grip dynamometry, digital grip dynamometry, box and block test before, after, at 1 month, and at 6 months
	2	61, F	spinal stenosis at C5-C6	D	C1	3 y		
	3	42, M	haemangioma at C4-C5	D	C4	15 y		
	4	61, F	arteriovenous malformation at T4-T5	D	C5	7 y		
	5	68, M	spinal stenosis at C3-C7	D	C4	8 y		
		3 males, age 42-68, mean 56						

1-month follow up. Motor function was assessed with MMT and MEPs. Additionally, EMG of some muscles innervated by the stimulated nerves was recorded in the paraplegic patient. Sensory score was evaluated before and after the stimulation; neuropathic pain was additionally evaluated at the follow up.

5.4.2.2 STUDY V

Study V explored PAS in 5 chronic traumatic SCI patients. A double-blind randomized sham-control study was designed. In 5 SCI patients, PAS/50 was administered to a randomly selected hand; the contralateral hand received sham TMS combined with PNS. The intervention lasted 4 weeks and included 16 stimulation sessions. PNS was administered using the same parameters as in the PAS protocol. Sham TMS was delivered with a TMS coil separated with a 75-mm plastic block placed between the TMS coil and the patient's head. TMS intensity was the same as in actual TMS in PAS. PAS and sham-PAS were performed in alternating order at every session. The main outcome measure was MMT evaluated before the start of PAS, after the intervention, and at the 1-month follow up. Spasticity score and neuropathic pain were evaluated at the same time. Sensory score, F-responses, and EMG were evaluated before and after the intervention.

5.4.2.3 STUDY VI

Study 6 explored the efficacy of PAS in neurological SCI. Five chronic tetraplegic SCI patients with different neurological origins participated in the study. PAS/100 was selected for the intervention as this was the most effective protocol obtained in study II (85). PAS was administered to the hand with lower MMT score for 6 weeks in 22 stimulation sessions. The contralateral hand was not stimulated. Patient performance was assessed before and after the intervention, and at 1-month and 6-month follow ups. Motor function was evaluated with MMT, functional tests, and hand dynamometry.

5.5 OUTCOME MEASURES

5.5.1 MEP

In studies on healthy subjects, the outcome was measured with an average of 30 MEPs (average of 10 MEPs in study I) up to 60 min after the intervention to evaluate sustainability of PAS effect. MEPs were elicited with TMS delivered at intensity of 120% RMT and sampled at a 3.3-s interval. MEPs contaminated with spikes in EMG activity during a 200-ms pre-stimulus interval were discarded from analysis to exclude influence of muscle preactivation on MEP size (86). MEP changes were calculated for each post-PAS evaluation as a ratio of post-to-pre-MEP average (MEP change = post-PAS MEP/pre-PAS MEP * 100%). A positive value indicated amount of MEP potentiation and a negative value indicated MEP depression.

In study IV, 30 MEPs were recorded from left ADM, left BR, and left and right APB in a tetraplegic patient and from left TA and the left gastrocnemius muscle (GC) in a paraplegic patient before, during, after the intervention, and at the 1-month follow up. MEPs were not evaluated in studies V and VI.

5.5.2 MMT

Motor score was assessed with the MMT. An experienced physiotherapist evaluated each hand and leg muscle separately (Table 1). Only muscles with a motor score below 5 at evaluation before the intervention were considered for overall analysis.

In study IV, the evaluating physiotherapist was familiar with the experimental setup but did not have access to the results of previous examinations. In studies V and VI, the physiotherapist did not know the experimental setup. An average of change in MMT was defined for each evaluation and MMT difference between the evaluations was calculated.

5.5.3 Spasticity

The same physiotherapist evaluated spasticity with the Modified Ashworth Scale on the same day as MMT (Table 6). The spasticity score was analysed in a similar way as the MMT assessment.

In study V, spasticity was additionally assessed from EMG signals. The number of spasticity-related spikes in the 500-ms interval preceding a MEP was counted visually in offline analysis. A spike event was identified as a sharp EMG activity exceeding the EMG baseline. The difference in number of spikes

Table 6 Modified Ashworth Scale.

score	description
0	no increase in muscle tone
1	slight increase in muscle tone of ROM
2	more marked increase in muscle tone through most of the ROM
3	considerable increase in muscle tone, passive movement difficult
4	affected part(s) rigid in flexion or extension

ROM, range of motion.

(after minus before) was used for statistics. Fifteen MEPs were used for the analysis of APB, ADM, and BR measured before and after PAS.

5.5.4 Sensory score

Sensory assessment was performed by a researcher familiar with the experimental setups according to the ASIA exam sheet (Table 2). Light-touch and pin-prick scores at the C2-T10 dermatomes (from C2-S5 dermatomes for a paraplegic patient in study I) were assessed bilaterally before and after the PAS treatment. A sum of sensory score was calculated for each patient and the difference between evaluations was analysed. In study VI, sensory function was not assessed, as studies IV and V did not reveal significant changes of sensory scores.

5.5.5 Neuropathic pain

Neuropathic pain was quantified with a Visual Analog Scale (VAS). VAS is a subjective measure of the amount of pain. The VAS is scored from 0 to 10, where 0 represents no pain and 10 the worst imaginable pain. Patients estimated the pain in VAS scale before and after the intervention and at the follow up.

5.5.6 Hand strength and dexterity

The patient sat straight in a chair during the tests. The arm was adducted and the elbow was in 90˚ flexion. Patients were instructed to keep their body

and arm position unchanged during the test. Each test was performed three times and the best score in kilograms was used for analysis.

Evaluation of mechanical hand grip strength was performed with an Extra™ Hydraulic Hand Dynamometer (North Coast Medline, Inc., USA). The device handle was adjusted to the patient's hand for comfortable task performance. Pinch dynamometry was performed with a Baseline® Mechanical Pinch gauge (Fabrication Enterprises Inc., USA) for evaluation of key pinch, tip pinch, and palmar pinch. In addition to mechanical dynamometry, digital dynamometry was performed with the rehabilitation device PABLO (Tyromotion GmbH, Austria). The initial patient position was the same as for mechanical dynamometry. However, measurement of grip and pinch force was encouraged with assistant movements of the body during digital hand dynamometry. Therefore, digital dynamometry expressed comprehensive performance of the task, reflecting hand ability in everyday life.

Unilateral manual dexterity was evaluated by the Box and block test (BBT). The BBT consists of two equal compartments divided with a separation wall and blocks that are collected in one compartment before task performance. The task is to transfer blocks from one compartment to another over the separation wall with one hand during a 60-s period. The result is the number of the transferred blocks.

5.6 DATA ANALYSIS

All statistical analyses were performed with SPSS 22.0-25.0 software. The data are presented as mean ± standard error (SE).

Data from all patient studies were analysed with Wilcoxon signed-rank test. In healthy-subject studies, amplitudes and latencies from an average of 30 MEPs of each evaluation were evaluated. Data were assessed for normality with the Kolmogorov-Smirnov test. The data were non-parametrically distributed except in experiment 2.1. Non-parametric data were analysed with Wilcoxon signed-rank test (study II, experiment 2.2, and study III) to compare the averages of MEP amplitudes pre-PAS vs post-PAS within one PAS protocol. To detect differences between PAS protocols, Kruskal-Wallis test (study I) and Friedman test (experiment 2.2, study II) were applied. In experiment 2.1, the data were distributed normally and ANOVA with repeated measures and Bonferroni post-hoc correction was applied.

6. RESULTS AND DISCUSSION

6.1 STUDY I: A modified PAS protocol is effective in a range of ISIs

ISI determination is crucial for PAS outcome and is often challenging in SCI patients as they have altered physiology in the corticospinal tract and spike-contaminated EMG. This study tested the efficacy of the modified PAS protocol with different ISIs to identify the most feasible PAS settings for clinical application.

6.1.1 Results

MEPs of all subjects in all the tested PAS protocols (ISI ±10 ms; ±5 ms, 0 ms) increased significantly immediately after the intervention with no difference between the protocols: 196 ± 23% at ISI -10 ms, 179 ± 17% at ISI -5 ms, 166 ± 31% at ISI 0 ms, 196 ± 14% at ISI +5 ms, 189 ± 9% at ISI +10 ms ($p = 0.043$ for all protocols). PNS and TMS delivered separately had no effect on MEP amplitudes (98 ± 11%, $p = 0.69$ for PNS, 94 ± 10%, $p = 0.89$ for TMS) (Figure 6). For individual averages of MEPs, see study I, Table 2.

6.1.2 Discussion

All PAS protocols induced a robust MEP potentiation. This contrasts with findings from studies that employed a conventional PAS protocol in healthy subjects (64,75) and SCI patients (63). Taylor JL and Martin PG (2009) tested seven PAS protocols at different ISIs ranging from -15 ms to +20 ms in healthy subjects and observed an LTP-like effect in MEPs induced only by the PAS protocol where the estimated activations of presynaptic and postsynaptic membranes were nearly synchronous, whereas the other six protocols induced depression of MEPs or did not have any effect (64).

Notably, PAS with ISI of 0 ms potentiated MEP less than the other four protocols. This may be explained by the high intraindividual variability to PAS depending on non-controlled subjective factors (see paragraph 7.3). It is also probable that a high-frequency PNS train employed in the modified PAS protocol enables prolonged postsynaptic depolarization and provides a larger time window for spike coincidence than the classical PAS protocol.

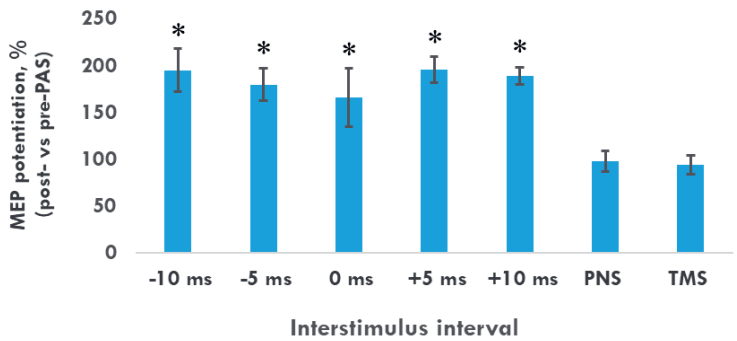

Figure 6 MEP potentiation induced by PAS protocols with different ISIs measured immediately after PAS. A significant increase of average MEP amplitudes at all tested protocols was observed. Only PNS and only TMS did not induce MEP change. Adapted from Shulga et al, 2016 (study I). The original publication is distributed under the terms of the Creative Commons Attribution License.

Accordingly, presynaptic activations for all non-synchronizing ISIs (±5, ±10 ms) may fit into LTP and LTD time windows preceding following postsynaptic activation induced by a high-frequency PNS train. If both LTP and LTD interactions exist in the -20 to +10 ms time range, LTP overtakes LTD (73) (Figure 7). This suggests that the present PAS protocol regulates ISI-dependent outcome such that PAS enhanced MEPs at a wide range of ISIs.

All stimulation protocols were performed with motor imagery of the targeted movement. Since motor imagery lowers the motor threshold (87) frequently elevated in SCI patients, this approach probably increases the efficacy of TMS and PAS protocols. One control PAS protocol with ISI of 0 ms, conducted without motor imagery, elicited a similar MEP potentiation as the other PAS protocols. It is probable that cortical activation induced by TMS at the highest possible intensity in our PAS protocol exceeds the effect of motor imagery at least in healthy subjects. Nevertheless, performing motor imagery during PAS in SCI patients could be useful as they often have diminished corticospinal transmission. Motor imagery might also involve secondary motor areas into synchronous activation induced by PAS.

ES is widely used during conventional rehabilitation for neurological patients. Substantial evidence indicates that ES modifies MEPs. However, the efficacy of PNS depends strongly on chosen parameters such as frequency, intensity, pulse waveform, and duration of stimulation (88). The control experiment with PNS analogous to those in the present PAS protocol had no

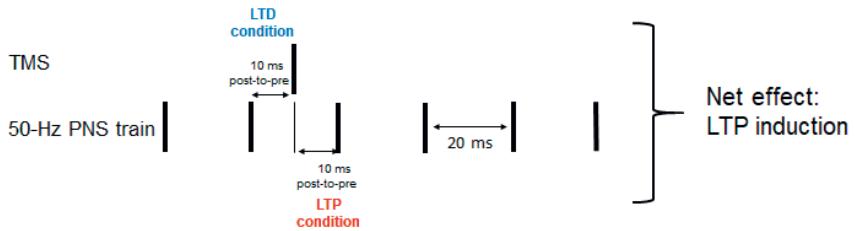

Figure 7 An example of TMS-PNS pairing in our PAS protocol under non-synchronous conditions. A single TMS pulse does not align with any of the 6 pulses of the PNS train. Instead, the TMS pulse drops between two subsequent PNS pulses. When TMS pulse is considered to interact with the preceding PNS pulse; LTD induction is expected; LTP induction is expected with the following PNS pulse. When LTP and LTD conditions are implemented at the same time, the net effect of this neuronal interactions results in LTP (73).

effect on MEP amplitudes. TMS alone also does not modify MEPs at the group level. This is consistent with observations of the effects of low-frequency (≤ 1 Hz) rTMS that suppresses or has no effect on corticospinal excitability (89,90). Chen et al. applied 0.1-Hz TMS at suprathreshold intensity for 1 hour and showed no MEPs changes, whereas a 0.9-Hz TMS protocol suppressed MEPs (91). It is thus unlikely that the 0.2-Hz TMS and PNS used in our PAS protocol could alone contribute to MEP potentiation after PAS.

Overall, our PAS protocol is designed to produce multiple descending and ascending volleys in the CST and seems to establish more complex interactions between volleys than the conventional PAS. It reduces influence of strict spike-timing on PAS outcome as demonstrated by results of the study I. This feature renders some freedom with respect to ISI determination and thus makes PAS feasible for application in SCI patients.

6.2 STUDY II

6.2.1 Experiment 2.1
Increasing frequency of PNS train potentiates PAS outcome

This study compared the effectiveness of PAS protocols with different frequencies of PNS train.

6.2.1.1 RESULTS

All three tested PAS protocols significantly increased MEP amplitudes at all time points as assessed by ANOVA with repeated measures with a Greenhouse-Geisser correction for multiple comparisons ($F(1.076, 31.210) = 9.488$, $p = 0.004$). A significant difference was observed between PAS/100 and PAS/50 ($p = 0.009$, 158 ± 25% 100 Hz vs 50 Hz at all time points) and PAS/100 and PAS/25 ($p = 0.016$, 151 ± 17% 100 Hz vs 25 Hz) and no difference between PAS/25 and PAS/50 protocols as measured with pairwise comparisons by Bonferroni post-hoc analysis. PAS/100 induced the strongest potentiation at all time points. In contrast, control PNS/100 did not show MEP potentiation at any time point (Figure 8).

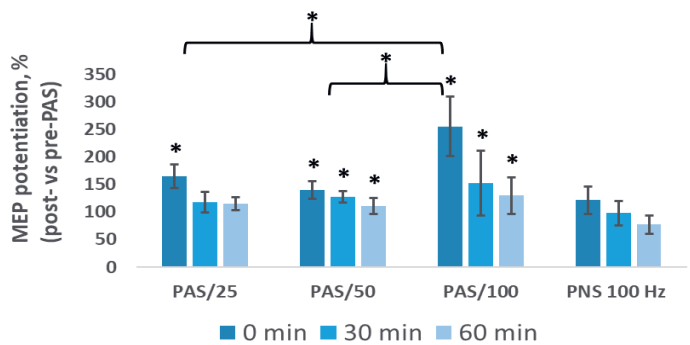

Figure 8 MEP potentiation induced by PAS protocols with PNS of 25 Hz, 50 Hz, and 100 Hz. PAS with 100-Hz PNS displayed the highest and most sustainable MEP potentiation. PNS only did not change MEP amplitudes. Adapted from Tolmacheva et al, 2019 (study II). The original publication is distributed under Creative Commons Attribution 4.0 International License.

6.2.1.2 DISCUSSION

In this study, PAS protocols with a PNS train at lower (25 Hz) and at higher (100 Hz) frequency than the previously used 50-Hz PNS were tested in 10 healthy subjects. Results revealed that a PAS protocol with a 100-Hz PNS train induced the strongest MEP potentiation at all time-point evaluations.

Stjöström et al (2001) implemented a multifaceted approach in biophysical cell models to elucidate mechanisms of STDP (73). They demonstrated that temporal correlation of pre- and postsynaptic activations is not the only determinant of the existence and polarity of STDP. STDP is also influenced by firing rate, excitatory postsynaptic potential (EPSP) size, amount of postsynaptic depolarization, and input cooperativity. A low-frequency induction protocol (such as our 0.2-Hz PAS protocol) does not generate LTP in the absence of sufficient EPSP, but LTP can be rescued with an additional postsynaptic depolarization. Such depolarization could be generated by a high-frequency PNS train in our modified PAS protocol. High stimulation frequency may generate stronger postsynaptic depolarization. LTP magnitude correlates positively with the size of postsynaptic depolarization (73). Hence, a high-frequency PNS train would yield a strong postsynaptic depolarization, resulting in more effective PAS. Indeed, PAS/100 induced stronger and more persistent MEP potentiation.

TMS administered at 100% of MSO intensity in a PAS protocol may also contribute to MEP potentiation. Suprathreshold TMS activates a large area of the cerebral cortex. In addition to M1, somatosensory and premotor areas may also be activated depending on the orientation of the TMS coil. Moreover, different conduction times along corticospinal pathways originating from different cortical areas account for variability in the timing of descending volleys. Furthermore, fast- and slow-conducting corticospinal neurons add additional dispersion in the arrival time of the volleys to the spinal cord. The corticospinal neurons respond to a single TMS with variable number of D- and I-waves and can thus result in different patterns of presynaptic activation (92). Hence, it appears that TMS activates CST in a complex fashion. Interaction of TMS- and PNS-induced volleys in CST cannot be predicted precisely in humans. Therefore, a simple approach to LTP induction based on an STDP model when only spike timing is considered does not permit fulfilling the potential of PAS. Moreover, high dispersion in the central and peripheral conduction time in CST of neurological patients must be considered when designing a PAS protocol. Importantly, LTP is more sensitive to spike-timing, EPSP size, and postsynaptic depolarization. In contrast, LTD is induced in a wider time window (-75 to 5 ms) than LTP (+5 to +25 ms) and does not require sufficient postsynaptic depolarization (73). Even a small shift in coincidence of single TMS- and single PNS-induced volleys could reverse polarity of outcome

in a conventional PAS protocol. High-frequency stimulation (>40 Hz) permits neglecting of spike-timing principle for LTP induction (73) and makes application of a PAS protocol with high-frequency PNS feasible in SCI patients.

6.2.2 Experiment 2.2
PAS with a suboptimal TMS target is rescued with high-intensity TMS

Cortical mapping in SCI patients is often challenging due to cortical reorganization. This study tested the efficacy of our PAS protocol when the M1 mapping is inaccurate.

6.2.2.1 RESULTS

A gain in MEP potentiation became significant on the third day of PAS by Wilcoxon signed-rank test for both the stimulated suboptimal spot (311 ± 141%, $p = 0.043$) and the adjacent sites (166 ± 26, $p = 0.043$). MEP amplitudes increased more on the third vs the first day in the stimulated suboptimal spot (184 ± 38%, $p = 0.043$) than in the adjacent area (147 ± 35%, $p = 0.043$), but the difference was not significant (Figure 9). Moreover, MEP amplitude increased significantly on the eighth vs the first day (measurement before PAS) only in the stimulated spot (209 ± 50%, $p = 0.043$ by Wilcoxon signed-rank test) (Figure 10).

6.2.2.2 DISCUSSION

M1 mapping is sometimes difficult in SCI individuals. M1 mapping is more complicated in severe than in mild SCI. The results demonstrated a gradual increase of MEP potentiation on the third day and the MEP baseline on fifth day after the 3-day PAS. TMS administered at 100% of MSO induces a wide E-field. Its maximum is located beneath the coil centre and field strength attenuates gradually outwards. Thus, TMS delivered to a suboptimal spot at 100% MSO also activates an adjacent area with lower intensity. Presumably, PAS induced stronger potentiation of MEPs corresponding to the stimulated suboptimal spot. This supports administration of PAS with TMS at 100% MSO in patients when precise M1 mapping is impossible. Three-day PAS also demonstrated a cumulative effect on MEPs, justifying long-term application of PAS to achieve a better outcome.

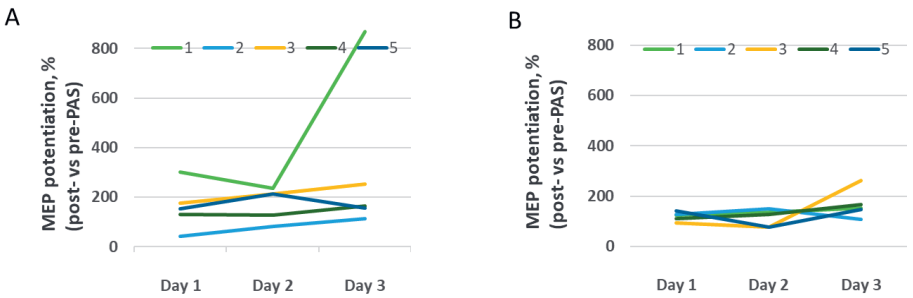

Figure 9 MEP potentiation is more effective in the stimulated spot. MEP potentiation of individual subjects measured from the stimulated spot (A) and the adjacent area (B). Adapted from Tolmacheva et al, 2019 (study II). The original publication is distributed under Creative Commons Attribution 4.0 International License.

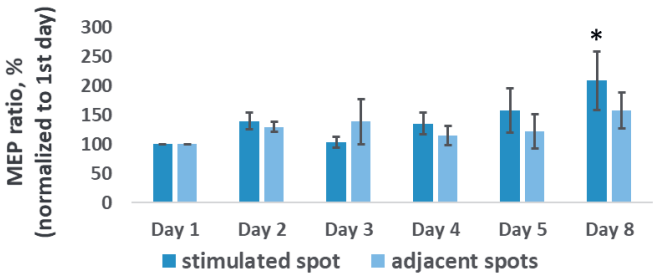

Figure 10 Greater increase of MEP baseline in the stimulated spot. MEP ratio from pre-PAS measurements during 3-day PAS and MEP measurements recorded subsequently up to the eighth day of the experiment, normalized to the first day. Adapted from Tolmacheva et al, 2019 (study II). The original publication is distributed under Creative Commons Attribution 4.0 International License.

6.3 STUDY III

6.3.1 Experiment 3.1
Increase of PAS frequency does not further enhance PAS outcome

A shorter stimulation session would make long-term PAS more convenient for SCI patients. This experiment compared the efficacy of a shorter 0.4-Hz PAS protocol with the originally used 0.2-Hz PAS protocol.

6.3.1.1 RESULTS

A 0.4-Hz PAS protocol increased MEP amplitudes immediately after the stimulation (204 ± 73%, p = 0.038). At 30 min after the session, a trend towards MEP increase (120 ± 10%, p = 0.066) was observed. MEPs returned to baseline at 60 min (103 ± 11%, p = 0.77). Our previously used standard 0.2-Hz PAS displayed a trend toward MEP increase (193 ± 43%, p = 0.66) immediately after the stimulation. MEPs increased significantly at 30 min (177 ± 24%, p = 0.008) and at 60 min (147 ± 10%, p = 0.008) (Figure 11).

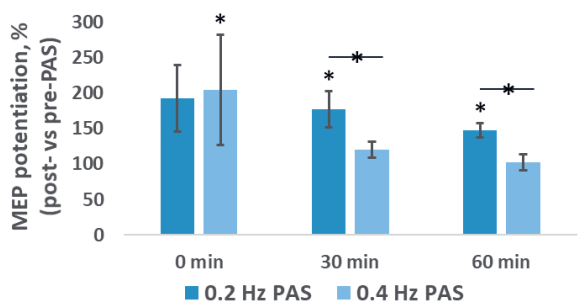

Figure 11 MEP potentiation after 0.2-Hz and 0.4-Hz PAS protocols. 0.2-Hz PAS induced greater and more persistent increase of MEP amplitudes. Adapted from Mezes et al, 2019 (study III). The original publication is under Creative Commons Attribution License.

6.3.1.2 *Discussion*

Both tested PAS protocols enhanced MEPs. This effect persisted longer after 0.2-Hz than 0.4-Hz PAS. The duration of the stimulation session and PAS frequency could both influence the results. In a classical PAS setup, a 30-min session at lower pairing frequency (0.05-0.01 Hz) potentiated MEPs up to 30 min (54,63). Consistently, 132 PAS activations delivered at 0.2 Hz over 11 min increased MEP amplitudes up to 20 min after the stimulation, whereas 90 PAS activations delivered at 0.05 Hz for 30 min did not elicit a significant MEP potentiation (93). A study that specifically examined the influence of PAS frequency demonstrated that plasticity is enhanced by the number of PAS pairs and by prolongation of the interval between them. The evidence suggests that frequency and total number of PAS pairs have more prominent impact on PAS outcome than the duration of the session per se.

This experiment revealed that our original 0.2-Hz PAS protocol used previously in patient studies is an optimal choice in balancing between therapeutic effect and duration of the stimulation.

6.3.2 Experiment 3.2

Increasing frequency of PNS train in PAS does not enhance PAS outcome.

In study II, 100-Hz PNS was more efficient than 25- or 50-Hz PNS in a PAS protocol. This experiment explored whether a further increase of PNS frequency enhances the PAS outcome.

6.3.2.1 *Results*

A PAS with 100-Hz PNS was compared with PAS protocols with 200-Hz PNS (PAS/200) and 400-Hz PNS (PAS/400). PAS/100 induced significant MEP potentiation immediately after (198 ± 25%, $p = 0.005$) and at 30 min after the stimulation (189 ± 28%, $p = 0.009$). The PAS/200 increased MEP amplitude immediately after (182 ± 22%, $p = 0.022$). PAS/400 did not potentiate MEPs. The PAS/100, PAS/200, and PAS/400 protocols differed significantly in between-group analyses of all time points. Post-hoc analysis revealed no difference between MEPs elicited by the PAS/100 and the PAS/200 protocols. The PAS/400 was less effective in enhancing MEPs than the PAS/100 and the PAS/200 protocols (Figure 12).

6.3.2.2 *Discussion*

Increasing PNS frequency from 100 Hz to 200 Hz and to 400 Hz did not enhance the efficacy of PAS in potentiating MEPs. The possible mechanism of high-frequency PNS trains to advance postsynaptic depolarization does not seem to contribute solely to PAS outcome (see paragraph 6.2.1.2). Additional coincidences between multiple TMS-induced volleys and multiple pulses of 200- and 400-Hz PNS trains did not enhance the PAS outcome. Lack of further growth in MEP potentiation could be explained by mechanisms of BDNF release triggered by elevation of intracellular calcium important in LTP induction. Cellular studies suggest that BDNF release depends on stimulus pattern and augments LTP progressively when stimulation frequency increases from 5 Hz to 50 Hz but with no added effect for 100 Hz (94-97). Absence of subsequent enhancement of LTP at frequencies greater than 100 Hz could reflect modification of BDNF secretion. This might explain the smaller MEP potentiation in PAS protocols with PNS at higher frequencies. In addition, the limited efficacy of the PAS/200 and the PAS/400 could be explained by the shorter time window for volley coincidence provided by these protocols.

Thus, PAS with a 100-Hz PNS train is the optimal choice out of the protocols tested so far.

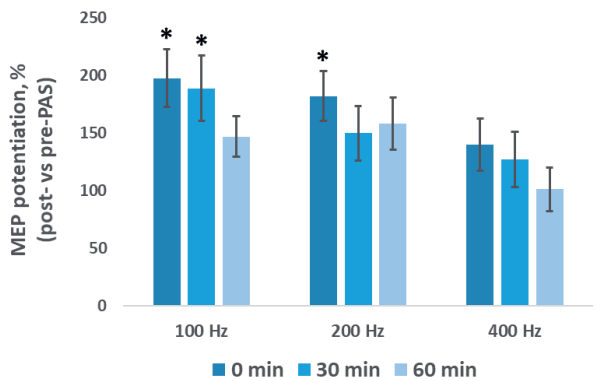

Figure 12 MEP potentiation after PAS with 100-Hz, 200-Hz, and 400-Hz PNS. PAS with 100-Hz PNS displayed stronger and longer MEP potentiation. Adapted from Mezes et al, 2019 (study III). The original publication is under Creative Commons Attribution License.

6.3.3 Experiment *3.3*
PAS with 20-Hz TMS inhibited corticospinal transmission

A paired-pulse TMS could increase the probability of coincident neuronal events in the spinal cord and could augment PAS outcome.

6.3.3.1 RESULTS

PAS with a paired-pulse TMS suppressed MEPs at all time points of the follow up. MEP amplitudes decreased significantly by -64 ± 14% ($p = 0.043$) immediately after, by -66 ± 20% ($p = 0.23$) at 30 min, and by -52 ± 12%, ($p = 0.043$) at 60 min (Figure 13).

6.3.3.2 DISCUSSION

The rationale for selection of 20-Hz paired-pulse TMS was to keep TMS intensity as high as possible, since TMS delivered at 100% of MSO is a characteristic feature of our PAS protocol (see paragraph 3.4.1 TMS in PAS). According to TMS safety guidelines, a 20-Hz paired-pulse can be delivered at 96% of the MSO. This frequency enabled synchronization of two high-intensity TMS pulses with the pulses of a PNS train. A 20-Hz paired-pulse TMS

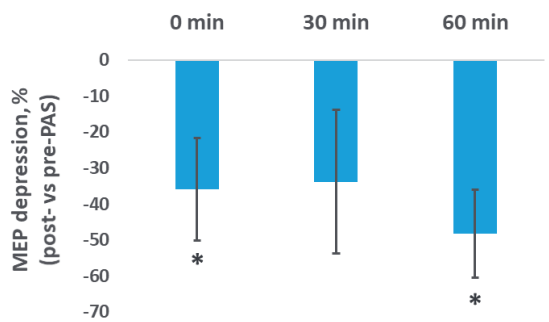

Figure 13 MEP depression after PAS utilizing 20-Hz paired-pulse TMS. The protocol induced lasting reduction of MEP amplitudes. Adapted from Mezes et al, 2019 (study III). The original publication is under Creative Commons Attribution License.

has a 50-ms interval between the two TMS pulses. Paired-pulse TMS given at intervals of 50-200 ms suppresses MEPs through long-interval intracortical inhibition (LICI) (98). However, the 50-ms interval is a borderline value for LICI and intracortical facilitation (ICF). For instance, Valls-Sole et al (1992) observed an increase of MEP amplitudes at double-pulse intervals of 25-50 ms and MEP suppression at intervals of 60-150 ms (99). The site of LICI action may involve both supraspinal and spinal inhibitory mechanisms. MEP suppression evoked by LICI protocols with intervals of 50-200 ms may relate to cortical modulation (100,101). Thus, the observed MEP suppression needs not to indicate spinal inhibition. It is probable that the induced LTP-like plasticity at the corticomotoneuronal synapses by PAS with a paired-pulse TMS is masked by a robust cortical LICI. However, I-waves are enhanced in epidural recordings despite MEP suppression after paired-pulse TMS delivered at a 50-ms interval. This MEP inhibition may involve a subcortical (likely spinal) mechanism (102). Although existing literature on mechanisms of LICI are ambiguous, a PAS protocol with a 20-Hz paired-pulse TMS induced MEP suppression and is therefore not useful in treatment of SCI patients.

6.4 STUDY IV: Long-term PAS restores some motor function in traumatic SCI

A single PAS session can facilitate transmission in the corticospinal tract associated with increased motor output in healthy individuals. This proof-of-principle study demonstrated for the first time the potential of PAS for motor rehabilitation when applied as a long-term intervention in two incomplete traumatic SCI patients.

6.4.1 Results

6.4.1.1 TETRAPLEGIC PATIENT

A tetraplegic patient received PAS to the right hand and control PNS to left MN for 12 weeks. The MMT score improved in the PAS-treated right hand already at 7 weeks after the onset of stimulation (on average by 0.5 points at 7 weeks and by 1.13 points at 12 weeks) and remained increased at the 1-month follow up. In contrast, the MMT score of muscles innervated by left MN did not change during the 12-week PNS (on average by -0.2 points at 7 weeks and

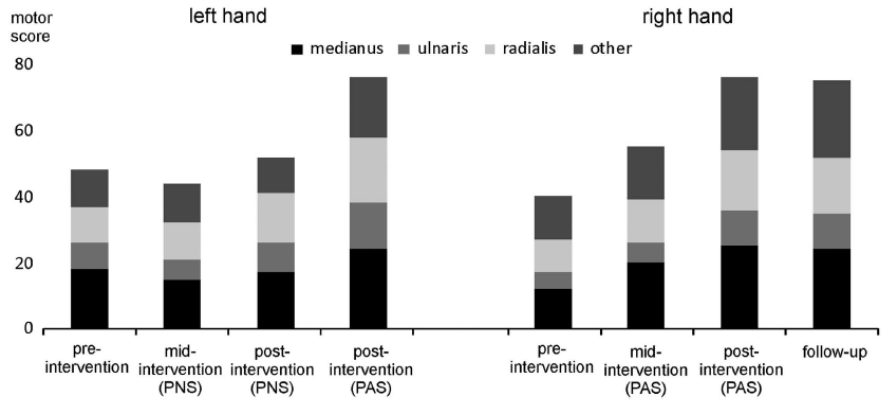

Figure 14 Motor score of a tetraplegic patient. Motor scores of muscles innervated by MN, UN, RN, and other nerves are presented separately. The changes in the left hand were not observed while receiving PNS. Motor score of both hands improved during PAS administration and at follow up. Figure from Shulga et al, 2016 (study IV). The original publication is distributed under Creative Commons Attribution 4.0 International License.

by 0.17 points at 12 weeks). However, after subsequent administration of PAS for 12 weeks to the left hand, the MMT score increased by 0.88 points. A smaller MMT score increase was also observed in the non-stimulated hand muscles. MEPs of left APB stimulated by PNS alone did not increase in amplitude (Figure 14). Ultimately, the improvement in muscle strength enabled grasping with the PAS-treated hand.

The sensory or spasticity scores did not change significantly. The sum of light touch and pin prick assessed in C2-T1 dermatomes for the right/left hand was 21/16 before and 20/14 after the intervention.

The patient reported less neuropathic pain during the intervention and follow up. Before the intervention, pain in the shoulder and scapular area occurred about 3 times per week; after the intervention, similar pain occurred only in the shoulder area once in 2 weeks.

6.4.1.2 PARAPLEGIC PATIENT

The paraplegic patient received PAS first to the left leg. At first, slight dorsi- and plantarflexion of the left ankle was observed in the left foot at 5 weeks after onset of PAS. During the first 9 weeks of PAS administered to the left leg, the

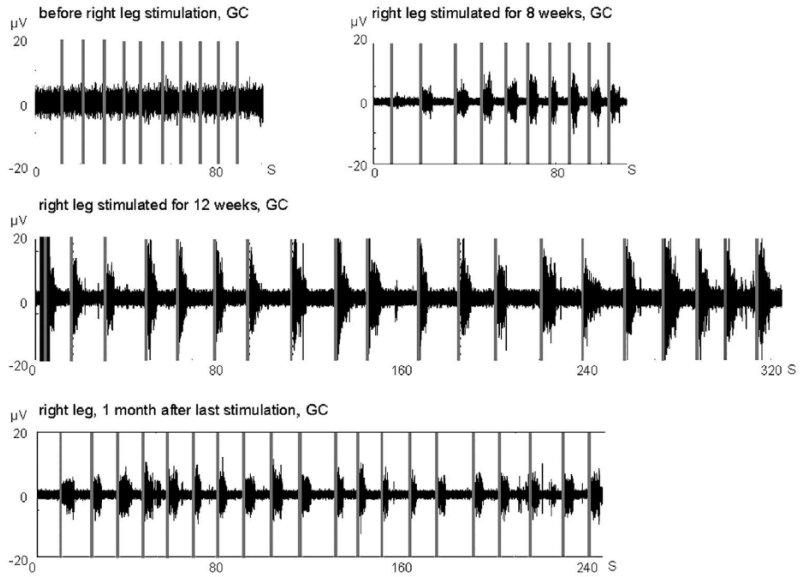

Figure 15 EMG recording from the medial GC in a paraplegic patient before, during PAS, and at follow up. Vertical dark grey lines represent a command for plantarflexion. Before PAS, no EMG movement-related activity is seen. Voluntary EMG activity appears at week 8 of PAS and continues growing over the intervention. Restored plantarflexion remains at 1 month after PAS termination. Figure from Shulga et al, 2016 (study IV). The original publication is distributed under Creative Commons Attribution 4.0 International License.

right leg was not stimulated and remained paralyzed. PAS of the right leg started at the tenth week. Voluntary movements in the right ankle were first seen after 3 weeks of stimulations. Voluntary activity of both feet were detected in EMG after 8 weeks of PAS; movement-related EMG activity and MEP amplitudes of stimulated muscles increased during the intervention and at the 1-month follow up (Figure 15). The L2-S3 dermatomes had abnormal sensory score before the intervention. The sum of light touch and pin prick from these dermatomes was 7 before the intervention, 8 at 8-week, and 9 at 20-week evaluations. Sensory scores did not change significantly after the intervention. The patient did not have spasticity before or after the intervention.

The patient had daily throbbing bilateral pain of VAS 3-6 in L2 dermatome before the intervention. During the intervention and at the 1-month follow up, the incidence of pain of VAS 4-5 dropped to a few times a week.

6.4.2 Discussion

The main result of the study for the tetraplegic patient was regaining the ability to grasp. The non-stimulated muscles also improved. It is plausible that changes in motor behaviour induced by PAS-related improvement of specific muscles engaged non-stimulated muscles, as they form a unitary functional motor system (103). This is consistent with observations on increased MEP amplitudes of non-target muscles after cortical PAS (54,76).

For the paraplegic patient, the main result was the appearance of subtle dorsi- and plantarflexion detected on EMG already at 8 weeks of PAS in previously paralyzed legs. The patient was not able to imagine dorsi- and plantarflexion before PAS treatment. During PAS, she first regained the ability to imagine the movements. The visible movements detected in EMG appeared after this.

Sensory scores were not modified significantly by long-term PAS. However, the paraplegic patient regained the ability to feel the acquired movements. This study was the first indication that long-term PAS with settings developed in our laboratory restored some voluntary control over previously paralyzed hand and leg muscles. The first improvement was already seen at 7 weeks after stimulation onset and increased until the end of intervention, suggesting that a longer period of stimulation may result in further motor improvement.

6.5 STUDY V
Long-term PAS improves motor function in traumatic SCI patients.

Study V compared for the first time the efficacy of long-term PAS to long-term PNS in traumatic SCI patients. PAS was delivered to one hand of the patients. The contralateral hand received only PNS. This setup ameliorated patient-related heterogeneity in medication, type of injury, and genetic factors in comparison of the effects of PAS and PNS interventions.

6.5.1 Results

The average MMT score of the PAS-treated hand increased by 0.74 ± 0.18 points ($p < 0.0001$). The average MMT score of the PNS-treated hand increased by 0.55 ± 0.08 points ($p < 0.0001$) immediately after the intervention. However, during the first month of follow up (change between “1 month” and “after” evaluations), the MMT score of the PAS-treated hand continued to increase whereas the MMT score of the PNS- treated hand did not

change. At 1 month, the MMT score increase was significantly higher in the PAS-treated hand (1.02 ± 0.17, $p < 0.0001$) than in the PNS-treated hand (0.65 ± 0.14 $p < 0.0001$) (Figure 16). The patients reported functional benefit of the intervention. For instance, patient 1 was able to open bottles and patient 3 was able to open doors with the PAS-treated hand.

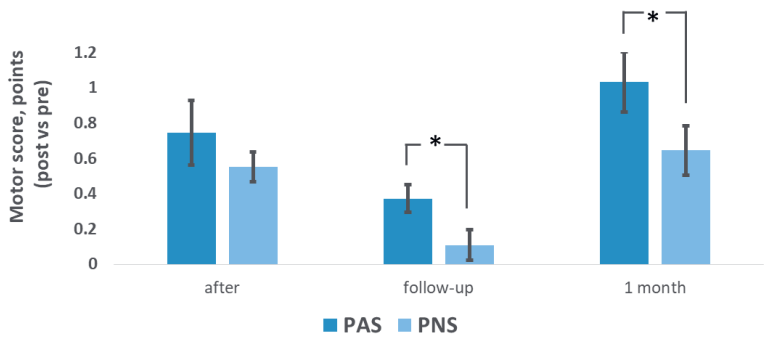

Figure 16 MMT score improvement. Average MMT score increase in muscles innervated by stimulated nerves. Adapted from Tolmacheva et al, 2017 (study V). The original publication is distributed under Creative Commons license.

Spasticity and EMG were not modified by PAS and PNS. The number of spasticity-related spikes in EMG recorded from APB, ADM, and BR decreased by 33 ± 35 spikes in the PAS-treated hand ($p = 0.24$) and by 23 ± 16 spikes in the PNS-treated hand ($p = 0.4$).

The sensory score from dermatomes C2-T10 did not change after the intervention. The difference between the assessments before and after PAS was -0.8 ± 1 point ($p = 0.46$) for light touch and 3 ± 1.8 points ($p = 0.14$) for pin-prick scores in the PAS-treated hand and 1.2 ± 0.6 ($p = 0.1$) for light touch and -1.4 ± 2 ($p = 0.7$) for pin-prick scores in the PNS-hand.

PAS and PNS did not modify F-responses measured from the UN and MN before and immediately after the intervention.

Three patients did not have neuropathic pain before or after the intervention. Patient 1 had unpleasant feelings in the right arm and feet, which disappeared after the intervention. Patient 5 had daily pain of VAS 3 in both forearms before the intervention; after the intervention the pain increased to

VAS 4-5 and returned to baseline level after the follow up. This change in pain score is probably related to interruption of regular peripheral stimulation for the period of the intervention and follow up.

6.5.2 Discussion

Although the MMT score increased immediately after the intervention in both hands, only the PAS-treated hand continued to improve during the follow up. PNS-induced MMT improvement could be explained by training of stimulated muscles (104). Motor imagery leads to descending activation along the CST (105). Hence, PNS combined with motor imagery may provide conditions for associative synaptic plasticity at the spinal cord and may contribute to MMT score improvement. However, synchronization of motor imagery with PNS is difficult to control and precise timing of descending and ascending activities required for STDP is improbable. The latency and persistence of F-responses did not change after the intervention, suggesting that peripheral nerve changes did not contribute to observed motor improvement. Changes in spasticity did not explain the MMT improvement by PAS.

This study provided the first direct evidence of the superiority of long-term PAS over long-term PNS in chronic SCI patients of traumatic origin. The results of this study and the pilot study suggest that longer application of PAS could yield greater motor improvement.

6.6 STUDY VI
Long-term PAS enabled functional improvement in non-traumatic SCI patients.

Neurological SCI constitutes a considerable proportion of SCI. Due to different aetiology and a usually milder injury, the response to PAS in neurological SCI could differ from traumatic SCI. This study investigated the efficacy of long-term PAS in neurological SCI patients. In this study, PAS was administered to one hand and the contralateral hand did not receive any stimulation to detect possible motor improvement in the non-stimulated hand.

6.6.1 Results

After the intervention, the average MMT score of the PAS-treated hand increased by 1.4 ± 0.4 points ($p = 0.043$) immediately after PAS, by 1.6 ± 0.4

points (p = 0.043) at the 1-month, and by 1.7 ± 0.5 points (p = 0.043) at the 6-month evaluations (Figure 17). The MMT score of the non-stimulated hand also improved in 3 patients who had an abnormal MMT score before the intervention. However, the MMT score improved more in the PAS-treated hand than in the non-stimulated hand. The ratio of the PAS- vs the non-stimulated hand from 3 patients was 157 ± 27% after the intervention, 129 ± 12% at 1 month, and 130 ± 9% at 6 months. The MMT score increase was accompanied with improvement in functional tests and hand strength in the stimulated but not in the non-stimulated hand. This was reflected in more confident use of the stimulated hand in everyday tasks such as dressing, hair washing, playing guitar, and handling a steering wheel.

Palm pinch, key pinch, and box and block tests improved at all evaluations, whereas tip pinch improved at 1 month evaluation, and digital dynamometry at 1- and 6-month evaluations. Consistent with previous patient studies, the spasticity score did not change in either hand.

6.6.2 Discussion

Motor improvement achieved by PAS in both hands persisted up to 6 months. The average MMT score increase was larger in patients with neurological than traumatic SCI. However, direct comparison of PAS efficacy is hampered by differences in PAS protocols and heterogenous patient groups. Patients with more recent SCI improved more. Outcome was followed up to 6 months and revealed a MMT score increase after PAS termination. This probably relates to more active use of the hands in everyday life due to acquired improvement during PAS. The MMT score of the non-stimulated hand also increased. Increased use of the PAS-treated hand could favour assistance of the contralateral hand during bilateral tasks. Moreover, interhemispheric and interspinal interactions are present in innervation of the hands. The cortical changes followed by SCI are characterised by enhanced activation in the primary somatosensory cortex and supplementary motor area, which may cause elevated interhemispheric inhibition to the less injured side (106). Thus, PAS-associated recovery of the stimulated hand leading to some restoration of interhemispheric balance could contribute to recovery of the non-stimulated hand.

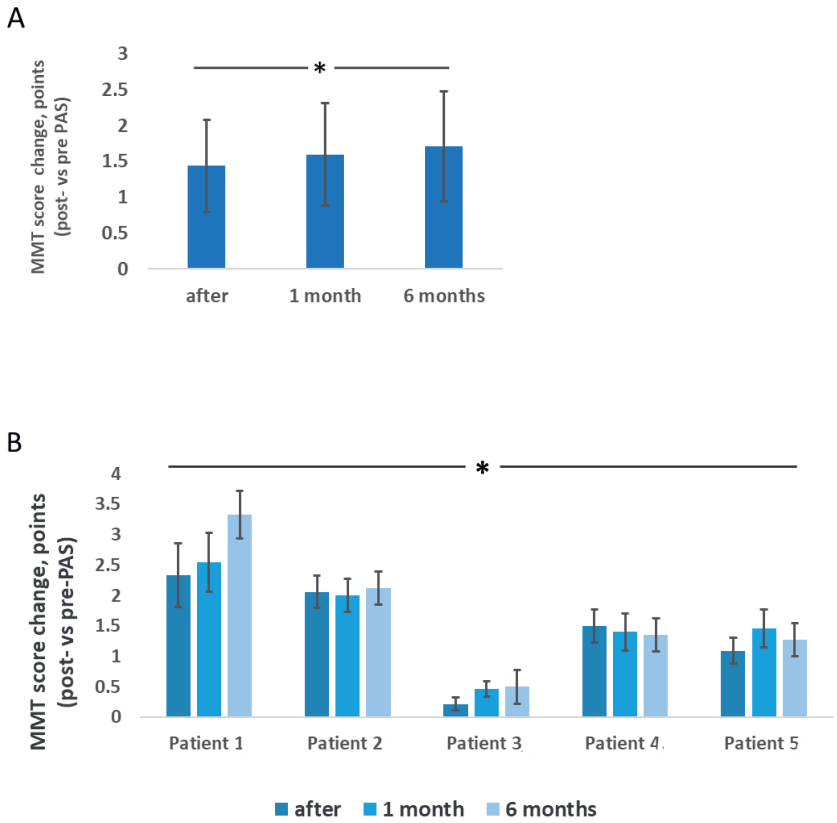

Figure 17 MMT score change after PAS in the muscles innervated by the stimulated nerves. (A) Average MMT score increase. (B) Individual MMT. Adapted from Tolmacheva et al, 2019 (study VI). The original publication is distributed under Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND).

7 GENERAL DISCUSSION

7.1 LIMITATIONS OF THE STUDIES

7.1.1 PAS outcome measured with MEPs

PAS outcome was measured with MEPs in studies I, II, and III. MEP used as a global measure of corticospinal excitability is somewhat limited in interpretation of the PAS outcome. A conventional MEP study does not define the level of CST contributing to MEP amplitude change. An after-PAS increase of MEP amplitudes can be attributed to modulation at the spinal cord and in the cerebral cortex.

Modulation of cortical circuits affects MEP amplitudes. A control experiment with TMS delivered alone in study I revealed no significant MEP changes, suggesting that TMS alone does not explain the PAS effect. Corticospinal transmission can also be influenced by cognitive factors during MEP measurements, which are difficult to control. For example, thinking of a movement facilitates corticospinal transmission plausibly by diminishing intracortical inhibition (83,107). Focused thinking on topics different from movement may reduce the TMS-elicited MEP amplitudes (108). The pattern of motor-neuron discharges in the spinal cord in response to TMS-induced pulses also influence MEP size. The number of recruited lower motor neurons, the number of motor neurons with repetitive discharge to the TMS stimulus, and synchronization of discharge of different motor neurons to the given TMS stimulus can modify MEP size (108). The number of recruited motor neurons is sensitive to background muscle contraction. Concurrent voluntary contraction is a powerful tool to enhance corticospinal excitability; even contraction of 5-15% of the maximum force level results in maximum MEP amplitudes. Analysis of continuous EMG during MEP measurements in our studies excluded this factor. For MEP elicited by high-intensity TMS, repetitive discharge of motor neurons is a relevant factor, as TMS evokes multiple descending volleys that causes motor neurons to fire more than once. A large between-subject variability in multiple activation of pyramidal cells to a given TMS stimulus does not enable precise estimation of the rate of descending volleys in each individual (108). Hence, this factor cannot be standardised during MEP measurements. Because motor neurons independently fire in response to a TMS stimulus, MEP size can be reduced by one-third due to

phase cancellation of desynchronized motor neurons; a negative phase of some motor-unit potentials is neutralized by a positive phase of the others (109).

Applying TMS to CST at the cervicomedullary junction enables assessment of corticospinal integrity at the spinal level (110-112). In contrast to MEP evoked by TMS, cervicomedullary stimulation does not activate corticospinal pathways selectively, as cervical TMS at MT intensity activates a large volume of the spinal cord involving multiple corticospinal pathways.

F- and H-response studies could be used for assessment of changes at the spinal cord. An F-response study could examine the excitability of a motoneuronal pool via antidromic activation. H-response is considered as an electrophysiological equivalent of the monosynaptic tendon reflex and reflects segmental motor excitability. Thus, F- and H-responses are not representative of the corticomotoneuronal synapses that are the targets in our PAS. Consistently, no change of F-responses was observed after spinal PAS (63).

MEPs were not a reliable method for outcome evaluation in patients due to spike-contaminated EMG, which confounds interpretation of MEP amplitude changes. A conventional MEP study would not provide exclusive information on efficacy of the corticomotoneuronal synapses. However, MEPs are broadly utilized for assessment of corticospinal excitability. It is a sensitive, objective, and feasible test. Focal TMS stimulation of M1 enables assessment of excitability of specific corticospinal pathways that are targets in PAS. Additional paired-pulse TMS studies could be used for estimation of cortical excitability to resolve a contribution of cortical modulation after spinal PAS.

7.1.2 Patient recruitment

SCI patients who have contraindications to TMS cannot undergo PAS. Patients below 18 years or over 75 years were not considered for PAS. We did not recruit patients who had SCI over 15 years ago or patients with congenital SCI, as these patients are not expected to be sufficiently responsive to PAS. Drug and alcohol abuse, psychiatric disease, and tumours of any location were excluding factors. During long-term PAS, the skin under the stimulating electrodes for PNS should be monitored for damage, as SCI individuals have susceptibility to skin problems. However, skin damage was not observed in any studies of this dissertation. Comorbid diseases that may interfere with interpretation of results, such as neuropathy, severe spasticity, or severe arthrosis may also be exclusion criteria. Progressive SCI were not considered for PAS, as the naturally progressive course of disease would mask PAS effect. Patients treated with tendon-muscle and nerve-transfer hand surgery should not be considered for PAS, as the changed neuromuscular connections do not

enable reliable identification of the parameters related to the CST activation. Long-term intervention that requires regular visits may exclude patients who live far from the laboratory.

Although recruitment of patients with the same SCI classified by AIS is feasible, these patients would form a heterogeneous group by localization and extent of lesion. It is thus difficult to perform a study with an appropriate control for these characteristics. However, patients with chronic SCI serve as their own control, as no spontaneous recovery is observed at the chronic stage.

7.2 POTENTIAL PAS MECHANISMS

7.2.1 Action at the spinal cord

Our PAS protocol was designed to strengthen the corticomotoneuronal synapses of spared corticospinal connections in SCI individuals. Based on individual adjustment of PNS and TMS timing, PAS presumably exerted an action on the corticomotoneuronal synapses at the spinal cord. Our PAS protocol employed a pre-postsynaptic activation model of synaptic plasticity. Studies on spinal PAS evaluating PAS outcome with cervicomedullary stimulation and transcranial electrical stimulation also suggest involvement of the spinal cord in the observed MEP potentiation (63,64).

PNS adjusted for activation of motor fibres also activates the sensory tract. Activation of the sensory tract during PAS possibly implements additional relevant neuronal interaction in the spinal cord via the tendon reflex loop (H-reflex) (68). The H-reflex is evoked by activation of the Ia afferents that have an excitatory monosynaptic input to the homonymous motoneuron. Since latency of H-response nearly matches to the F-response latency, a calculated ISI in a PAS protocol would synchronize inputs from a motoneuronal axon, a corticospinal neuron, and Ia afferent to homonymous motoneuron (113). It is therefore conceivable that the intended pre-postsynaptic activation mechanism in our PAS is strengthened with an additional sensory input (18). This secondary mechanism may serve the goal of the study to augment corticospinal transmission.

7.2.2 Action at the cortical level

A sensory impulse ascends to the sensory cortex and synapses intracortically to M1. As such, our PAS protocol could actualize cortical PAS similarly to the original PAS protocol, which supposedly represented a

convergent model of synaptic plasticity (18,54). In our PAS protocol, the nearest interaction of PNS- and TMS-induced inputs to a pyramidal cell is provided by the first pulse of the PNS train and the latest I-wave. The delay of the converged inputs would be approximately 5 ms for a hand and 15 ms for a foot muscle, with TMS-induced inputs preceding the PNS-induced volleys. Subsequent pulses of PNS train and earlier I-waves preserve the same TMS-prior-PNS order but with a greater delay. This order of activation would lead to suppression of motor output (see 3.4.3 Interstimulus interval). High-intensity TMS could also activate the axonal hillock of the pyramidal cell. This could actualize a pre-postsynaptic PAS model via backpropagation of an action potential to postsynaptic dendrites of the pyramidal cell (18). Still, this type of neuronal interaction would probably induce LTD, since postsynaptic activation precedes presynaptic activation. Thus, at first approximation, one could argue for a supplementary action of our PAS protocol, resulting in suppression of M1 output. However, our PAS protocol enhanced MEPs in all experiments. This suggests that either our PAS protocol induced a modest cortical effect that was overridden with a targeted potentiation in the corticomotoneuronal synapses or that other neuronal interactions occur at the cerebral cortex. This is also supported with results where long-term PAS restored movement-induced modulation of sensorimotor spontaneous activity that was accompanied with neurological improvement in SCI patients (Vanhanen et al, submitted). However, it is questionable whether the restored sensorimotor activity modulation was due to a direct effect of PAS on the cerebral cortex or developed secondarily as a response of the sensorimotor system to PAS-induced changes in the spinal cord.

Considering PNS and TMS timing in our PAS protocol, it is likely that our PAS protocol does not have a sole operant mechanism. Previous experiments on long-term synaptic plasticity have been conducted in cell models. These studies revealed timing rules for LTP and LTD induction and were used as a simplified model that enables prediction of PAS effects in humans. Determination of all possible pathways and neuronal interaction over non-invasive stimulation is rather challenging in humans. Accumulated knowledge on PAS in human studies has revealed facilitation of corticospinal output at a range of ISI (56,58). Potentiation of M1 projections to intrinsic hand muscles requires predominantly the order when a sensory input to the pyramidal cell precedes activation by TMS. For leg muscles, MEP potentiation can also be seen in a reverse order (58). These conclusions were drawn from studies that employed a classical PAS protocol; in contrast, our modified PAS protocol presumably grants flexibility in timing of neuronal interaction needed for LTP induction.

However, there is a high interindividual variability in responsiveness to conventional facilitatory PAS protocols, characterized by no effect or even suppression of the M1 output. Stable traits-like factors influencing PAS effect, such as age, neuroanatomical differences, and genetic factors have been determined. In addition, temporal state-related factors, such as preceding physical activity, medication, circadian fluctuation, and focused attention can change the PAS outcome (56).

7.3 BENEFITS OF A LONG-TERM PAS ADMINISTRATION

In healthy-subject studies, the efficacy of a single PAS session was demonstrated with MEP potentiation that was shown to correlate with improvement in motor function (63,64). It is therefore presumed that the transient neurophysiological effect of a single-session PAS is predictive of long-term behavioural effects. Study IV showed a progressive improvement of relevant measures during 21-24 weeks of PAS treatment. A first-ever progressive full restoration of hand muscle strength was described after 47 weeks of modified PAS in a chronic SCI patient (AIS B); the MMT increase was associated with considerable functional improvement in the SCIM score (115). Thus, duration of PAS influences the extent of achieved motor improvement. This could be attributed to the cumulative effect of PAS seen in experiment 2.2 and to an increased use of the hand once function has been regained during long-term treatment. Similarly, the patient's motor performance may improve beyond PAS treatment, as was observed in study VI. MMT increased more in patients with less severe non-traumatic SCI (AIS D) than in traumatic SCI patients (AIS B and C). However, two traumatic SCI patients with a comparable gain in MMT exhibited some functional improvement as well. This highlights that patients with less affected motor function could benefit functionally during 4-6 weeks of PAS. Most improvement was already seen during the first 2 weeks of long-term PAS. It is probable that for patients with low muscle strength at baseline, longer stimulation is required to achieve functional improvement (115).

Spontaneous recovery in the chronic stage of SCI is unlikely (114). Plastic changes in CST in response to neuromodulation interventions are not so prominent as during the first year since injury. However, all 12 chronic patients with heterogenic origin and severity of SCI, time since injury, and age responded to long-term PAS treatment. It is plausible that the observed motor improvement was due to PAS. All patients tolerated long-term PAS well and did not have PAS-related adverse effects.

7.4 PAIRED VERSUS UNPAIRED STIMULATION

The improbable contribution of 0.2-Hz TMS to change corticospinal excitability is discussed in study I (paragraph 6.1.2).

FES is effective in rehabilitation of motor function. A special FES setup could actualize the principles of associative synaptic plasticity at the spinal cord by inducing antidromic activation of motoneurons (21). However, this remains speculative, since principles of Hebbian rules require precise neuronal activations in the range of a few milliseconds. It seems implausible that voluntary contraction during FES could be adjusted by an individual with such accuracy. Moreover, PAS has an advantage over FES, as PAS can restore voluntary control over previously paralyzed muscles. Even a small motor improvement in muscle strength that enables regained function would be critical for the overall rehabilitation outcome.

In addition to the action of PNS itself, PAS bears an emergent property, induction of synaptic plasticity, which results from conditioned neuronal interactions. This unique feature of PAS may advance this technique over single-mode stimulation. Indeed, study IV and V demonstrated the superiority of long-term PAS over long-term PNS. In study IV, long-term PNS did not increase MMT score when applied to MN, although subsequent administration of PAS to three hand nerves of the same hand increased MMT score. The effect of PNS administered to only one nerve may not be sufficient to induce motor improvement in our patient with severe SCI.

In study VI, motor improvement was also observed in the non-stimulated hand. The contribution of PNS to the observed motor improvement in study V cannot be separated from the bilateral effect of PAS. To investigate therapeutic potential of PNS with parameters paralleling our PAS protocol, long-term PNS was delivered to one hand in chronic SCI patients with similar clinical patient characteristics as in study VI. The MMT score increased after long-term PNS and was sustained at a 1-month follow up. However, the level of a MMT score increase was substantially lower in the PNS-treated hand (average 0.59 points) than in the PAS-treated hand in study VI (average 1.44 points) and was not associated with functional improvement (116). Nonetheless, long-term PNS with the parameters used demonstrated effectiveness in motor improvement (although less than long-term PAS) and can be offered as rehabilitation for an SCI patient with contradictions to TMS or when TMS is not available.

In this dissertation, 0.2-Hz PAS/100 was developed empirically in series of experiments with the goal to find more effective settings. Indeed, all

experiments of this dissertation employing 0.2-Hz PAS/100 induced MEP potentiation immediately after the stimulation in all healthy subjects (total 13 subjects and 20 sessions), whereas the other tested PAS variants did not. This suggests that this variant of PAS protocol enables reliable facilitation of corticospinal transmission and works well in SCI individuals.

7.5 FUTURE DIRECTIONS OF PAS

The motor system is functionally and anatomically reorganized after SCI. Although the epicentre of injury is in the spinal cord, excitability of cortical circuits after SCI is also altered (78,117). Corticospinal output is reduced by impairments of both cortical and spinal circuits. Accordingly, enhancement of cortical output may elevate efficacy of spinal PAS. As such, facilitatory rTMS could be employed in the spinal PAS protocol. Moreover, long-term rTMS itself may induce favourable plasticity at the cerebral cortex and contribute to restoration of corticospinal transmission.

Individuals in the acute or subacute stage of SCI could respond to PAS treatment better, as spontaneous recovery can be promoted with PAS. Patients with more recent SCI benefited more in studies V and VI. Moreover, early start of rehabilitation may prevent muscle atrophy and spasticity, which worsen the overall condition of people with SCI.

PAS could be combined with other rehabilitation approaches. For instance, PAS can be enhanced during vagus nerve stimulation (VNS). Intensive training concurrently with precise VNS results in a considerably higher level of motor recovery in chronic neurological patients (117-120). Because PAS is capable of targeting plasticity in specific circuits, PAS could be combined with cell therapy to prevent unwanted sprouting and to guide plasticity (121).

The parameters of a PAS protocol (duration of stimulation, frequency of PAS, PNS and TMS parameters) and a schedule of the treatment can be optimized further in PAS interventions.

8 SUMMARY AND CONCLUSIONS

The goals of this dissertation were to investigate the therapeutic efficacy of long-term PAS and to optimize the PAS protocol. These goals were achieved in the six studies.

1. Parameters of the PAS protocol influence PAS efficacy. A comprehensive approach in the assessment of parameters of our modified PAS protocol enabled detection of the optimal settings. A variant of PAS 0.2 Hz employing a single TMS at 100% MSO and 100-Hz PNS train is the most effective PAS protocol tested so far.
2. Long-term PAS had a therapeutic effect and demonstrated safety and feasibility in chronic SCI patients of different ages, SCI severity and pathogenesis, and time since injury.
3. Long-term PAS was more efficient than long-term PNS.
4. Long-term PAS is capable of restoring hand functions. Longer PAS administration generates a better functional improvement.

PAS treatment has demonstrated superiority over ES, which is broadly used as conventional rehabilitation in SCI. PAS presumably strengthens residual connections of CST in the spinal cord that serve as a substrate for motor recovery after injury. Modification of our PAS protocol enabled resolving issues relevant for SCI patients that were encountered in the classical PAS protocol. Our long-term PAS was effective in all patients. This promising result justifies further investigation of long-term PAS in larger cohorts of patients to assess the full potential of this method. Many laboratories are equipped with PNS and TMS devices that can be utilized for PAS rehabilitation of SCI patients.

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